BACTERIAL INFECTION IN CASES OF PERINATAL DEATH

A morphological and bacteriological study based on 264 autopsies

BY LARS OLDING

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From the Swedish by Helen Frey

GEFLEBORGS TR. A-B. GÄVLE 663795

To my parents

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CHAPTER 1

INTRODUCTION

Morison (1963) has recently reviewed the previous research on infection in the perinatal period of life, and so this will only be briefly touched upon here.

Every compilation of statistics on perinatal mortality gives infection as a major cause of death during this period. Morison (1952), for example, reported that infection was responsible for about 30 per cent of deaths within a month after livebirth (for about 20 per cent of deaths within three days and about 50 per cent of the later ones), and Ahvenainen (1959) stated that it accounted for 20 per cent of 1,247 deaths within a month after livebirth.

Figures of this nature do not show how many of all infants born get infected during this period of life. The children who die are often severely handicapped by conditions such as immaturity, severe malformation and cerebral hemorrhage, and so are probably more susceptible to infection than others. On the other hand, all newborn infants seem to be particularly liable to bacterial infection. Why they are, is not completely clear. One reason may be that they have no antibodies to E. coli (antigens O and H), because these are not transmitted from mother to child or only in small amounts (Adamsson, Löfgren & Malmnäs, 1951; Neter, 1955; Smith, 1960), yet E coli are often present in the upper respiratory passages shortly after birth (Smith & Bloomfield, 1950; Laurell, 1952), and they predominate in cases of neonatal septicemia (Nyhan & Fousek, 1958, Buetow *et al.*, 1965; and others). Furthermore, while term infants show about the same titers of antistreptolysins and antistaphylolysins when they are born as their mothers (Adamsson *et al.*, 1951; Vahlquist, Lagercrantz & Nordbring, 1950; Smith, 1960) these antibodies do not seem to affect the clinical course of streptococcic and staphylococcic infection in the infants (Vahlquist, 1960).

Earlier morphological studies on perinatal infection have been mainly concerned with pneumonia and placentitis, and the connection between the two. Hess—Thaysen (1914) seems to have been the first to observe the occurrence of pneumonia in the stillborn and the liveborn dying shortly after birth. Pneumonia at this time of life, he said, was characterized by the absence of fibrin and the frequent occurrence of signs of aspiration of amniotic fluid. Johnson & Meyer (1925) found this type of pneumonia, or congenital pneumonia as they called it, in 19 per cent of stillborn in-

fants and liveborn infants dying within three days. Later authors found rates between 12 and 15 per cent in stillborn infants (Macgregor, 1939; Barter, 1953; Morison, 1963).

The cause of pneumonia in the stillborn and neonatal infant is subject to debate. Warwick (1934) found bacteria in the lungs of 1 out of 19 stillborn infants with pneumonia. Macgregor (1939) got negative cultures from 10 stillborn infants with pneumonia and signs of prenatal aspiration. Bernstein & Wang (1961) got positive bacterial cultures from 34 out of 55 infants with pneumonia who died during the first week of life; like Macgregor, they found mostly coli bacteria and nonhemolytic streptococci in the bacterial flora. Douglas & Stander (1943) got positive blood cultures from 83 out of 225 stillborn infants (38 per cent); 73 out of the 83 were autopsied, but only 13 of them had pneumonia. Penner & McInnis (1955) got positive cultures from 33 out of 36 perinatally dying infants. Smith, Jennison & Langley (1956), examining 10 dead infants born to mothers with pyrexia during delivery, got positive lung cultures from 9; 4 of them had pneumonia.

Some authors have assumed that some cases of pneumonia in the stillborn and newborn infant are not caused by infection but by matter inspired into the lungs before delivery (Johnson & Meyer, 1925; Warwick, 1934; Cruickshank, 1949; and others).

More than thirty years ago, Johnson & Meyer (1925) drew attention to the frequent combination of congenital pneumonia with inflammation in the fetal membranes, and their observation was confirmed by Morison (1952), who collected the two reactions under the name of "syndrome of infection of the amnion". Blanc (1959) found a highly significant correlation between these two types of inflammation, and called the combination the "amniotic infection syndrome".

The types of inflammation occurring in the fetal membranes, placenta and umbilical cord have been described by several authors, and there are several reports on the frequency of the different kinds. Benirschke & Clifford (1959), studying 1,300 consecutive cases, found inflammation in the chorion and amnion (chorio-amnionitis) in 12.7 per cent, and vasculitis in the umbilical cord in 10 per cent. Later Benirschke (1960) reported the following observations in 1,536 consecutive cases: inflammation in the chorion only (chorionitis) in 19 per cent, inflammation both in the chorion and amnion (chorio-amnionitis) in 13 per cent, vasculitis in the umbilical cord in 11 per cent, and inflammation in both the membranes and umbilical cord in 9 per cent. Dominguez, Segal & O'Sullivan (1960), studying the umbilical cord in 500 deliveries, found vasculitis in 24.8 per cent.

The causes of inflammation in the placenta, membranes and umbilical cord are also subject to debate. Most authors blame it on infection, and some studies support this. Thus Benirschke (1960) and others demonstrated that the frequency of inflammation increases with the length of time between rupture of the membranes and delivery, and with the duration of labour, and Gosselin

(1945) demonstrated that bacteria are apt to invade the amniotic fluid when there is a lapse of six hours or more between rupture of the membranes and delivery.

Non-infectious causes have also been suggested for placentitis, such as voiding of meconium before birth, and changes in the pH of the amniotic fluid. Blanc (1961) and Bourne (1962) have recently given extensive reviews of the research on the appearance and causes of placentitis, and this subject will not be taken up in any detail here.

The connection between inflammation of the placenta and bacterial infection in the child is still obscure. Engström & Ivemark (1960) got positive blood cultures from the umbilical cord in 4 out of 40 cases in which the placenta and umbilical cord were both inflamed; only 2 of the 4 infants had shown any clinical signs of infection. They also got positive cultures from the umbilical blood in 3 out of 102 cases without any inflammation in the placenta or umbilical cord. Kobak (1930) got positive cultures from the blood in the umbilical cord in 9 per cent of 374 consecutive deliveries, but he only examined the placenta histologically when the blood cultures from the umbilical cord were positive or the delivery was protracted.

Only rarely have authors made both morphological and bacteriological examinations of several organs from consecutive series of infants dying during the perinatal period. The first to make a penetrating study of this kind was Quensel (1899); he found bacteria in 128 out of 144 infants, 29 of whom were less then 3 months old though none were under a week old—streptococci in 52 per cent, staphylococci in 18 per cent, B. coli in 13 per cent, and diplococci in 11 per cent. Rhomer *et al.* (1954) made a similar comprehensive study of 50 liveborn infants dying within 30 days; 50 per cent of the infants dying within 24 hours after birth had completely negative organs, apart from the occasional intestine; all the infants surviving more than 24 hours gave positive bacterial cultures; according to these authors' criteria for infection, 58 per cent of their 50 infants were infected.

—oOo—

The following is a report of a study of bacterial infection among infants dying during the perinatal period of life. It is based on the morphological and bacteriological observations in 264 cases—123 of stillbirth and 141 of death within 28 days after livebirth.

The main questions arising in a study of this kind are: How should one define bacterial infection? What morphological criteria should one use for bacterial infection, and why? How much can one rely on the results of postmortem culture for revealing bacterial infection? What sources of error are inherent in cultures made postmortem, and how much do they affect the results of culture?

To throw light on these questions, the study was planned as follows: (1) definition of bacterial infection; (2) definition of different morphological lesions and a study of their occurrence in the series; (3) study of the bacteriological results obtained, and analysis of the sources of error that might affect

the results of culture by means of statistical calculations, and a separate experimental study; and (4) comparison between the morphological and bacteriological observations, to try to determine the morphological signs of bacterial infection in this category of infants.

CHAPTER 2

DEFINITIONS

Bacterial infection

Not all authors define infection the same way. Dorland's Medical Dictionary (1965), for example, defines it in one place as "Invasion of the body by pathogenic microorganisms and the reaction of the tissues to their presence and to the toxins generated by them; often applied to presence of microorganisms within the tissues, whether or not this results in detectable pathologic effects," and in another place as "a general term applied to invasion of the body by bacteria, protozoa and helminths." Rosebury & Sonnenwirth (1958) said that "All that it (infection) implies is an interaction of a host organism with a smaller self-replicating agent such that both are modified in the process" and "disease is only one of several possible consequences of infection."

Space does not allow a discussion on the apparent or real differences between these definitions. From a practical standpoint and in view of the methods of examination used in the present study, the definitions in Dorland's dictionary suit this study best, both being applicable.

"Pathogenic" microorganisms are ones that cause disease. During the first few days of live, however, the clinical signs of infection are often vague and nonspecific, and often only autopsy can establish the diagnosis of infection. The autopsy diagnosis, in turn, can only be based on morphological and bacteriological criteria, and the pathogenicity of the bacteria evaluated only from the bacteriological standpoint. There is hardly any question about the pathogenicity, or potential pathogenicity, of some bacteria during the perinatal period; examples of these are beta-hemolytic streptococci, pneumococci, Listeria, and coagulase-positive strains of Staphylococcus aureus. The pathogenicity at this time of life of the bacteria "indigenous to man" is harder to judge; according to Rosebury & Sonnenwirth (1958), examples of these are coliforms, enterococci, Staphylococcus albus, Alcaligenes fecalis, nonhemolytic streptococci, and lactobacilli. As these authors pointed out, these bacteria present "a continuous spectrum from . . . imminent or potential pathogenicity . . . through apparently permanent neutrality . . . to symbiosis." This circumstance is of particular significance during the perinatal period, when the organism meets these bacteria for the first time, and when it

is highly susceptible to bacterial infection.

The coliform bacteria differ to a certain extent from the other indigenous bacteria. During recent years they have been found in ever increasing proportions among the bacteria yielded in neonatal septicemia (Nyhan & Fousek, 1958; Buetow *et al.*, and others). Furthermore, the newborn lack antibodies to E. coli (chap. 1). Thus, during the perinatal period at any rate, coliform bacteria must be regarded as at least potentially pathogenic.

It is also conceivable that bacterial invasion of an organism does not always give rise to morphological lesions demonstrable with conventional histological methods, or morphological lesions not yet recognized to be of infectious origin. Moreover, when an infant is stillborn, it may be so macerated that it is impossible to make a thorough histological analysis.

Consequently, when describing how much infection one finds in a series, one must first say what criteria one uses for infection.

For describing the results of the present study, I have used two definitions for bacterial infection:

(1) Infection in both the morphological and bacteriological sense, i. e., histological changes in the organs indicative of bacterial infection, together with positive bacterial cultures.

(2) Infection in the bacteriological sense only, i. e., no morphological changes indicative of bacterial infection, but positive bacterial cultures.

The cases of bacterial infection in either of these senses were divided according to whether the bacteria isolated were (a) pathogenic or (b) of indeterminate pathogenicity.

Analyses of the correlations between the morphological and bacteriological changes were used for choosing the morphological signs of bacterial infection, main emphasis being placed on the morphological changes other authors have taken as pointing to bacterial infection, i. e., different types of inflammation.

The pathogenicity of the bacteria was judged from the experience collected up to date in practical, clinical bacteriology in infancy (p. 19). The number of positive organs in each subject was noted, as several infected organs support the diagnosis of infection from the bacteriological standpoint.

Livebirth and stillbirth

According to Swedish law, a liveborn infant is one "who breathes or shows other signs of life at birth;" for what may be considered signs of life, the practice has been to follow the recommendations of the World Health Organization (1950). A stillborn infant, according to the law, is one who does not show these signs of life at birth, and who dies after 28 or more completed weeks of gestation. Products of conception dying before 28 completed weeks of gestation (fetuses), are not entered in the official registers. When it is uncertain how long the gestation has proceeded before the child died, it is registered as a stillborn infant if it is 35 cm. long or more, no matter what it

weighs. In the present study no distinction was made between infants and fetuses.

The subjects in the present study were classified as stillborn or liveborn in the University Department of Obstetrics.

Perinatal periods I and II

The terms perinatal period I and perinatal period II are used here as defined by the American Medical Association in 1959 (Potter, 1961). Thus deaths in perinatal period I begin with deaths of infants weighing more than 1,000 g and include deaths in the first 7 days after livebirth, and deaths in perinatal period II begin with deaths of infants weighing more than 500 g and include deaths in the first 28 days after livebirth.

Degrees of maceration

The infants were classed as *slightly macerated* when they showed incipient epidermolysis—a few vesicles on the skin or loosening of the epidermis in a few places—but still had a firm cranium and showed no hemolytic discolouration of the skin. They were classed as *greatly macerated* when they showed extensive epidermolysis, discolouration of the skin from hemolysis, a flabby cranium, and products of maceration in the body cavities. The reason the maceration was divided into slight and great was mainly that the organs of the slightly macerated can still be evaluated histologically, at any rate as far as gross lesions like inflammation are concerned, whereas the organs of the greatly macerated can not.

CHAPTER 3

MATERIAL AND METHODS

Material

Infants

According to official statistics, more than 99 per cent of all Swedish children are born in a hospital.

The present series comes almost entirely from the University Hospital in Uppsala. This hospital is the medical centre in the county of Uppsala, which has about 175,000 inhabitants. About 80 per cent of all the deliveries in the county take place at the Obstetric Department of this hospital. The others take place in two smaller hospitals, but these send mothers showing severe complications of pregnancy, or expected to have a difficult labour, to the University Hospital, as well as all liveborn babies born prematurely or with a severe disorder. The University Hospital also serves a region larger than the county of Uppsala for cases of Rh incompatibility, severe maternal diabetes, and severe malformation in the infants.

During the four years 1959—1962, 9,556 infants were born in 9,453 deliveries at the Obstetric Department of the hospital: 9,353 singletons, 98 pairs of twins, 1 set of triplets and 1 set of quadruplets. Altogether 207 of these infants died during perinatal period I (2.2 per cent), and 204 of these (98.6 per cent) were autopsied.

Altogether 269 infants dying during perinatal period II at the University Hospital in these four years were autopsied. It is not possible to tell exactly from the clinical records how many deaths took place in perinatal period II during these years, because small stillborn subjects are not always weighed in the hospital, length being used to distinguish between infants and fetuses (see chap. 2). But there is reason to assume that nearly all the products of conception dying during perinatal period II during these years were autopsied.

Table 1 shows all the 269 infants born and dying at the University Hospital autopsied during these four years, grouped according to whether they died within perinatal I or II, and by sex.

The *main series*, as it will henceforward be called, consists of infants examined both histologically and bacteriologically postmortem. They amounted to 264—154 males and 110 females. These comprised:

(1) 218 infants born at the University Hospital in Uppsala during 1959—1962, either stillborn or dying within 28 days after birth; these accounted for 81 per

cent of the original 269 born and dying perinatally in Uppsala during these years. Table 1 shows how many of the 218 died within perinatal period I and how many within perinatal period II, and how they were divided by sex.

(2) 32 liveborn infants (20 boys and 12 girls) born at another hospital, but referred to the University Hospital where they died before their 29th day of life.

Thus altogether 250 of the 264 subjects in the present series died at the University Hospital.

(3) The remaining 14 of the 264 were infants dying at hospitals outside Uppsala and sent to the Institute of Pathology shortly after death. They consisted of 9 stillborn (5 boys and 4 girls) and 5 liveborn (3 boys and 2 girls) dying within 24 hours after delivery.

Table 2 shows all the stillborn in the main series divided by birth weight and degree of maceration, and table 3 the liveborn divided by birth weight and length of survival.

As seen from table 1, postmortem cultures were not made from 51 of the consecutive series of 269 infants born in Uppsala. Most of these infants were autopsied during the short periods I was away from the Institute during the four years in question, at which times no cultures were made of this category of infants.

The 51 noncultured Uppsala-born infants accounted for 19 per cent of the altogether 269 autopsied Uppsala-born infants dying within perinatal period II. (The percentage was the same for the infants dying within perinatal period I.)

Twenty-six of the 51 were stillborn and 25 liveborn. This proportion about corresponds to the proportion between stillborn and liveborn in the whole autopsy series of Uppsala-born infants (table 1). The 26 noncultured stillborn included 9 nonmacerated, 3 slightly macerated and 14 greatly macerated infants. All except 1 of the 25 noncultured liveborn died in the first three days of life.

Twelve of the noncultured 51 weighed between 500 and 1,000 g at birth, 24 between 1,001 and 2,500 g, and 15 more than 2,500 g. This means that 23 per cent of the altogether 52 Uppsala-born infants belonging to the first weight class were not cultured, 20 per cent of the 119 in the second weight class, and 15 per cent of the 98 in the third weight class.

TABLE 1. *Autopsied infants*[a] *born and dying at the University Hospital of Uppsala during 1959—1962, by perinatal period within which they died, and by sex*

Figures in brackets denote number also examined bacteriologically

	Perinatal period I			Perinatal period II		
	Male	Female	Total	Male	Female	Total
Stillborn	69 (55)	43 (37)	112 (92)	86 (69)	54 (45)	140 (114)
Liveborn	56 (43)	36 (31)	92 (74)	72 (57)	57 (47)	129 (104)
Total	125 (98)	79 (68)	204 (166)	156 (124)	109 (90)	269 (218)

[a] About 99 per cent of all infants dying during perinatal period I, and probably equally many of those dying during period II

TABLE 2. *The 123 stillborn infants in the main series by birth weight and degree of maceration*[a]

Figures in brackets denote number born in Uppsala

Birth weight in grams	No maceration	Slight maceration	Great maceration	Total
501—1,000	10	2	10	22 (22)
1,001—1,500	5	—	9	14 (14)
1,501—2,000	4	3	7	14 (13)
2,001—2,500	8	2	5	15 (14)
>2,500	28	10	20	58 (51)
Total	55 (51)	17 (17)	51 (46)	123 (114)

[a] As defined on p. 13

TABLE 3. *The 141 liveborn infants in the main series by birth weight and length of survival*

Figures in brackets denote number born in Uppsala

Length of survival	Birth weight in grams 501—1,000	1,001—1,500	1,501—2,000	2,001—2,500	>2,500	Total
0— 6 hrs.	11	8	11	7	12	49(39)
7—12 hrs.	2	4	2	2	5	15(12)
13—24 hrs.	3	4	3	2	4	16(13)
25—48 hrs.	1	6	1	2	4	14(12)
49—72 hrs.	1	3	2	—	1	7(5)
4— 7 days	2	6	3	2	4	17(10)
8—28 days	1	2	—	2	18	23(13)
Total	21(18)	33(25)	22(14)	17(15)	48(32)	141(104)

Altogether 37 infants not born in Uppsala died at the University Hospital of Uppsala during the four years in question. Cultures were not made from 5 of these, 4 of whom died within the first three days of life.

Placentas

Altogether 164 placentas from the infants born at the University Hospital were examined histologically, 113 of them bacteriologically as well. It was not possible to examine the placenta in the other cases for reasons beyond my control. One hundred and nine came from 111 stillborn infants, including 2 monochorionic sets of twins with a common placenta and 2 dichorionic sets of twins with separate placentas. The other 55 came from 56 liveborn infants, including 1 pair of monochorionic twins with a common placenta and 3 dichorionic quadruplets with separate placentas (the 4th quadruplet is not included in the series.)

Besides these placentas from the cases of perinatal death, the placentas of 46 surviving children were examined. These placentas were collected by taking every tenth one delivered during two short periods in 1961 and 1962. Two of the placentas collected in this order turned out to belong to stillborn infants, and are not included among the 46. The 46 placentas were examined in the same way as the ones belonging to the infants who died.

Methods

The stillborn were placed in a refrigerator directly after they were delivered to await transport to the Institute of Pathology. The liveborn were transferred within two hours after death to the hospital morgue, where the temperature is kept at +10 C, or sent directly to the morgue at the Institute of Pathology, where the temperature is kept at +4 C. There were occasional exceptions among the liveborn dying during the night; then sometimes as much as eight hours might have elapsed before the body was put in the morgue, but it was always kept in a cool place in the ward in the meantime.

Removal of specimens for bacteriological examination

From infants. Before the body was opened, the whole anterior surface was disinfected with tincture of iodine or a solution of Desivon (quaternary ammonium base compound).

After this, the chest and abdominal cavities were opened with sterile instruments. These antiseptic precautions were taken because of the risk that the skin might still be contaminated by bacteria from the mother's vagina or rectum.

Next, pieces were taken from the organs *in situ,* using freshly sterilized sets of small scissors and forceps for each organ.

Specimens were removed in the following order. (1) Heart blood. After the pericardium was opened with sterile instruments, blood was withdrawn from the right ventricle with a syringe. This gave 0.5 ml or more in most cases, but when the body was macerated it was sometimes necessary to open the ventricle and take a specimen of blood with a cotton swab. (2) The lower lobe of the right lung. (3) The spleen. (4) The liver. A piece was taken from the right lobe, care being taken to avoid the gallbladder and biliary ducts. (5) The left kidney. To get to this organ, it was necessary to loosen the mesentery of the colon from the posterior abdominal wall, and push the intestine medialward. (6) The proximal section of the small intestine and colon. Pieces were ligated off and removed.

Large specimens of the various organs were removed; for example, half the pulmonary lobe was removed from the small babies, and half the spleen.

The surface of the removed tissue was flamed according to the method of Lossius (1941) and Adamsson (1949); thus the pieces were dipped in absolute alcohol, shaken to remove the excess alcohol, and set fire to by drawing them rapidly through an open flame. The pieces were put in sterile Petri dishes.

Adamsson (1949) studied how effectively this method sterilized the surface and how it affected bacteria in the tissue underneath. He dipped one set of pea-sized lymph nodes in different broth cultures of bacteria (Staph. aureus and beta-hemolytic streptococci) and using a fine needle inoculated another set with the same bacterial cultures, and then set fire to both sets. He found that the flaming removed all the bacteria from the surface of the tissue in the first set of nodes, but did not prevent direct culture of the bacteria under the surface in the other set. The flaming produced a ca. 50 micron deep zone of coagulation on the surface of the nodes.

When meningitis was known to be present or suspected, cerebrospinal fluid was taken in the following way. After the scalp was taken off and the roof of the skull disinfected with tincture of iodine, a syringe with a long, coarse needle was inserted through the anterior fontanel into the ventricles of the brain, care being taken to avoid the sagittal sinus. When the body cavities contained any inflammatory exudate, specimens were removed with cotton swabs.

From placentas. Immediately after delivery, the placentas were put in large, sterile Petri dishes and placed in a refrigerator. After a lapse of a few hours at the most, they were sent to the Institute of Pathology; there a large piece, taking in all the layers from the fetal surface to the decidua basalis, was removed from beside the site of attachment of the umbilical cord. This tissue was dipped in alcohol and ignited, and cultures were made of the tissue from the centre of the specimen, i. e., from the villous section. Pieces of the extraplacental fetal membranes, i. e., those not covering the placenta, were cultured without having been flamed first. Most of the placentas studied came from stillborn infants, but they also include a number from liveborn children transferred to the Pediatric Department directly after delivery because they were premature or had some severe disorder. In cases of this nature, the flamed pieces of the placenta were put in sterile Petri dishes and deep-frozen (—20 C). Then, if the child died shortly after birth, they were thawed out and cultured at the same time as the organs.

The placenta and fetal membranes were not cultured when it was obvious that they had been exposed to bacterial contamination, as in the case of manual detachment of the placenta or the "milk test" (in which milk is injected into the umbilical vein after the placenta is voided, to see whether there is any defect in the placental tissue).

The removed specimens were put in sterile Petri dishes and transferred immediately to the Institute of Microbiology, which is in the same building as the Institute of Pathology.

Culture

The flamed pieces of organs and placentas were divided with sterile instruments, and the fresh cut surfaces streaked on agar plates. For cultures in enrichment media, pieces were taken from the centre of the specimens. For study of Listeria, the pieces were homogenized first.

The samples were cultured on the following:

(1) Sheep blood agar plates
(2) Sheep blood agar plates containing gentian violet 1:750,000

(3) Hematin blood agar plates
(4) Sheep blood agar plates containing 2 per cent tryptose
(5) Agar plates containing 0.04 per cent potassium tellurite
(6) Liver-liver broth (Tarozzi)
(7) Dextrose broth containing thioglycollate
(8) Tryptose phosphate broth containing furacin (McBride *et al.*, 1960)

The first five were used for primary cultures and the last three for enrichment of bacteria. The plates were inspected three days in succession. At the beginning of the investigation the enrichment cultures were inspected for six days in a row, but when it was discovered how slowly Listeria sometimes grew, the period of observation was extended to 12 days. After 12 days of observation, secondary plate cultures (1—5) were made from the cultures in the enrichment media, even if they showed no growth.

During 1960 to 1962, while an epideciological study was being made of Listeria (Ekelund *et al.*, 1962), a new set of primary cultures were made after the tissue had been incubated at +4 C for a month, and the same procedure followed again.

Coliforms, staphylococci, streptococci and other bacteria isolated were identified according to current methods. Isolated coliforms were typed serologically against 12 types of enteropathogenic coli: 026:B_6, 055:B_5, 0111:B_4, 0114, 0119:B_{14}, 0125:B_{15}, 0126:B_{16}, 0127:B_8, 0128:B_{12}, 086:B_7, 0112ab:B_{13}, and 0112ac:B_{11}.

In 7 out of the 8 cases in which Listeria were grown, the strains were typed by Dr. Seeliger, Bonn. In 2 of the 3 cases in which beta-hemolytic streptococci were isolated, the strains were typed by Dr. Juhlin, Malmö.

All the bacteria found in the primary plate cultures were noted. Whenever bacteria occurred in the enrichment media that were not seen in the plate cultures, they were noted when they were pathogenic. As a rule it was not hard to identify the bacteria. When they were present, they appeared in most of the media used.

Evaluation of pathogenicity. The following bacteria found in the study were designated as pathogenic: coliforms (defined in this study as E. coli, Aerobacter aerogenes, Klebsiella pneumoniae), beta-hemolytic streptococci, pneumococci, Listeria monocytogenes, Staph. aureus and Salmonella. All the other bacteria discovered were judged to be of indeterminate pathogenicity.

Dr. G. Laurell of the Institute of Microbiology in Uppsala examined most of the bacterial cultures.

Autopsy procedure and removal of histological specimens

Every subject, whether or not it was macerated, was given a complete autopsy, and all its organs inspected grossly, according to the special technique we use at our institute for autopsying infants. This technique corresponds in all essentials with that recommended by Potter (1961).

Except for the rare case, I did all the autopsies myself und whenever not I re-examined all the histological slides from the case.

Besides specimens from organs showing gross lesions, specimens were taken routinely as follows: Samples of tissue were taken from both lungs, so as to permit histological examination of the hilus and the whole or a large part of the lung at the site of its largest cross-section. One specimen was taken from the mediastinum on a level with, or just below, the bifurcation of the trachea, and including the large bronchi and esophagus. From the heart was taken a specimen including the left ventricle, the septum, and the right ventricle. The spleen, liver and kidney were also examined routinely. Pieces were also taken of the proximal part of the small intestine and the colon. All the endocrine glands were removed for a separate study to be published later by Hultquist, Larsson and myself. But both adrenal glands were examined routinely for the present study. The brain and cerebral meninges were also examined histologically when infection of the central nervous system was present or suspected, or when they showed some form of obscure gross lesion.

The placentas, umbilical cords and fetal membranes were first inspected grossly, and then specimens were taken from the following sites: three levels of the umbilical cord, the placenta at the site of attachment of the cord and from the margin (generally from every quadrant) and from the extraplacental fetal membranes. The sections taken from the placenta took in all the layers, from the surface of the amnion to the decidua basalis.

The pieces of tissue removed were fixed in 10 per cent formaldehyde.

Van Gieson's and hematoxylin-eosin stains were used for routine staining. Special stains were used in some cases: Gram and Giemsa stains to demonstrate micro-organisms in the histological sections; Levaditi's method for cases of listeriosis; the Weigert stain to help identify fibrin, as well as Mallory's phosphotungstic acid hematoxylin stain (PTAH). In some cases the periodic acid-Schiff reaction (PAS) was also used.

Altogether about 5,000 histological slides were examined for the study.

CHAPTER 4

MORPHOLOGICAL LESIONS IN THE LUNGS AND PLACENTA

Lungs

The following section takes up morphological lesions observed in the lungs of the infants in the present study, with the exception of the greatly macerated stillborn, as it turned out that it was not possible to make a proper histological analysis in their case. Apart from these, it takes up the lesions observed in the lungs of all the infants autopsied, including the Uppsala-born subjects who were not cultured. The findings in the lungs of the consecutive series of Uppsala-born subjects are given separately in table 4, and the findings from the whole main series in table 5.

Prenatal aspiration of particulate matter

In the present study, as in most others of the same type, it was considered that the presence of particulate matter from the amniotic fluid or birth canal in the air spaces, or potential air spaces, indicated that the infant had inhaled before it was born. Matter of this kind includes vernix squames, vernix caseosa, meconium or mucus. Vernix squames and vernix caseosa and mucus are easy to identify in slides. When meconium is inspired, the pulmonary parenchyma often becomes tinged with green, and meconium bodies and sometimes bile pigment are seen in histological slides. In the present cases, the meconium bodies were generally mixed with vernix squames or vernix caseosa.

To begin with, signs of prenatal aspiration were graded as massive, moderate, or slight. With *massive aspiration* (fig. 1, 2) both the bronchi and alveolar ducts were filled with aspirated matter, and the whole or the greater part of the lungs were involved. When the child was stillborn, the potential air spaces were expanded, sometimes nearly as much as in the normally air-filled lung of the newborn. With *moderate aspiration,* about a third of the air spaces in the lung slides contained inhaled matter. It turned out, however, that it was hard to draw a distinct line between massive and moderate aspiration, and they were therefore grouped together. By *slight prenatal aspiration* (fig. 3) is meant only occasional small clumps of vernix squames or vernix caseosa in the alveolar ducts and alveoli, with the large bronchi free from foreign matter.

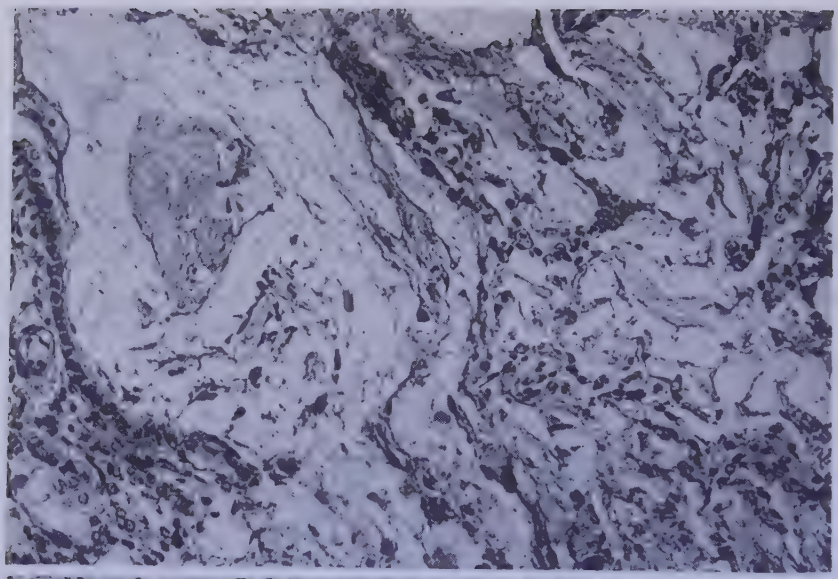

Fig. 1

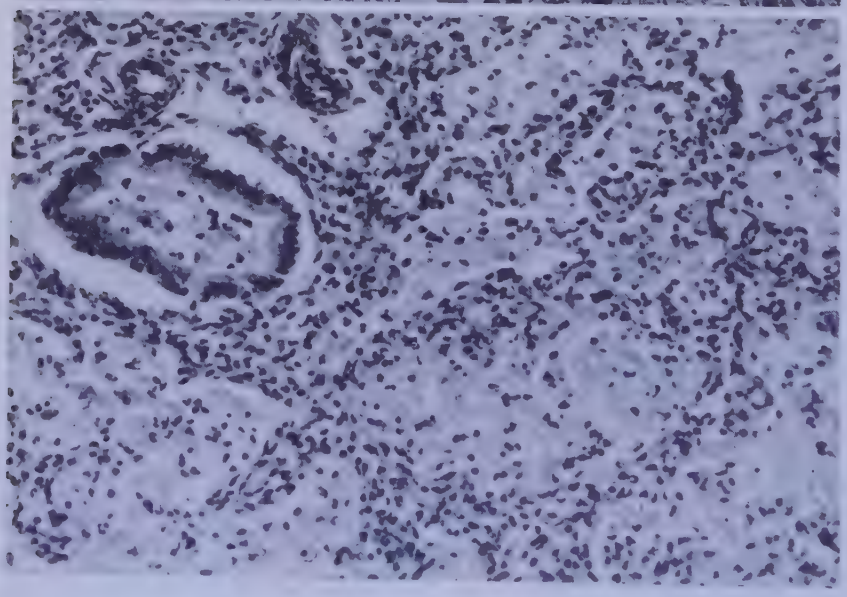

Fig. 2

Fig. 1. Massive prenatal aspiration of meconium plugging large bronchi. Lung cultures negative for bacteria. (*Liveborn, 3,600 g*) *PAS,* × *200.*

Fig. 2. Massive prenatal aspiration of vernix squames, combined with diffuse pneumonia. Lung cultures negative for bacteria. Placental membranes showed chorio-amnionitis. (*Liveborn, 800 g*) *H & E.,* × *200.*

Fig. 3

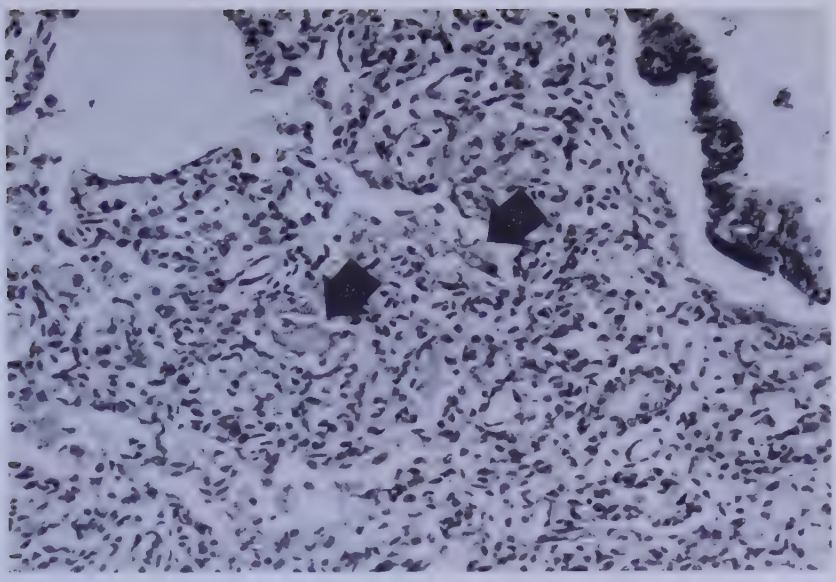

Fig. 3. Slight prenatal aspiration with vernix squames in occasional alveolar ducts and alveoli (arrows). Lung cultures negative for bacteria. (Liveborn, 1310 g) H & E., × 200.

Fig. 4

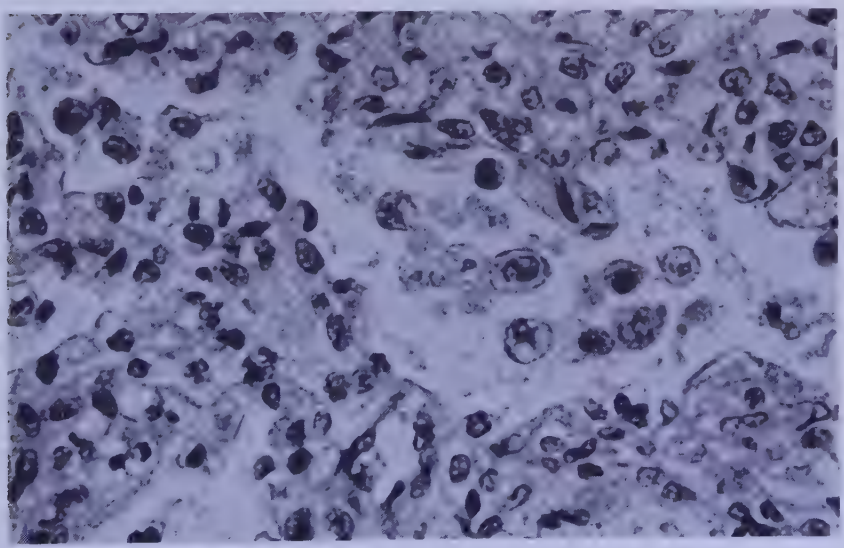

Fig. 4. Macrophages in alveolar ducts. Lung cultures negative for bacteria. (Liveborn, 980 g, dying four days after birth) PAS, × 450.

As seen from tables 4 and 5, in the present study signs of prenatal aspiration of some degree were observed in about 50 per cent of the nonmacerated or slightly macerated stillborn, and of massive or moderate degree in 35 to 40 per cent. The corresponding figures for the liveborn dying within three days were about 30 and 15 per cent. Most of the liveborn showing signs of massive or moderate aspiration died within 12 hours after birth.

Macrophages in the alveoli (fig. 4)

Whenever there were groups of macrophage-like mononuclear cells with an abundant, pale cytoplasm in the alveolar ducts and alveoli, the case was grouped under "macrophages in the air spaces". When these cells were present, the lungs often showed scattered areas of atelectasis, small foci of intra-alveolar hemorrhage, and interstitial edema.

It is obscure what causes these changes. One likely explanation is postnatal aspiration of foreign matter.

TABLE 4. *Various morphological lesions in the lungs. Cases of perinatal death in which the infants were born and died at the University Hospital of Uppsala during 1959—1962 (N=269) excluding greatly macerated stillborn infants*

The eight subjects whose lungs were not examined histologically were not included. No consideration is given to whether or not the infants were examined bacteriologically.

Morphological lesions in lung	Stillborn (N=78)		Liveborn							
			Dead within 3 days (N=99)		Dying on 4-7th day (N=11)		Dying on 8-28th day (N=12)		All liveborn (N=122)	
	No.	%	No.	%	No.	%	No.	%	No.	%
Prenatal aspiration										
All degrees	40	51	32	32	1	9	0	—	33	27
Massive or moderate	27	35	16	16	0	—	0	—	16	13
Pneumonia										
All kinds	12	15	29	29	2	18	6	50	37	30
Diffuse	4	5	8	8	1	9	5	42	33	27
Patchy	8	10	19	19						
Granulomatous	0	—	2	2	0	—	0	—	2	2
Hemorrhagic[a]	0	—	0	—	0	—	1	8	1	1
Septic infarction	0	—	0	—	1	9	0	—	1	1
Interstitial inflammation	17	22	26	26	5	45	9	75	40	33
Hemorrhage										
Extensive interstitial	16	21	9	9	0	—	1	8	10	8
Diffuse intra-alveolar	1	1	2	2	3	27	2	17	7	6
Hyaline membranes	0	—	27	27	0	—	0	—	27	22
Macrophages in air spaces	0	—	2	2	3	27	7	58	12	10

[a] also grouped under diffuse intra-alveolar hemorrhage

TABLE 5. *Various morphological lesions in the lungs. Main series (N=264) excluding greatly macerated stillborn infants*

Morphological lesions in lung	Stillborn (N=72)		Liveborn							
			Dead within 3 days (N=101)		Dying on 4-7th day (N=17)		Dying on 8-28th day (N=23)		All liveborn (N=141)	
	No.	%	No.	%	No.	%	No.	%	No.	%
Prenatal aspiration										
All degrees	39	54	31	31	3	18	2	9	36	26
Massive or moderate	29	40	15	15	0	—	0	—	15	11
Pneumonia										
All kinds	11	15	30	30	3	18	10	39	43	30
Diffuse	4	5	8	8	2	12	8	35	37	26
Patchy	7	10	19	19						
Granulomatous	0	—	2	2	0	—	0	—	2	1
Hemorrhagic[a]	0	—	1	1	0	—	1	4	2	1
Septic infarction	0	—	0	—	1	6	0	—	1	1
Interstitial inflammation	17	24	31	31	7	41	16	70	54	38
Hemorrhage										
Extensive interstitial	18	25	11	11	2	12	2	9	15	11
Diffuse intra-alveolar	1	1	4	4	3	18	2	9	9	6
Hyaline membranes	0	—	28	28	0	—	0	—	28	20
Macrophages in air spaces	0	—	2	2	6	35	13	57	21	15

[a] also grouped under diffuse intra-alveolar hemorrhage

Potter (1961) described what she called lipoid pneumonia i.e., a diffuse distribution of macrophages containing particles of fat in the alveoli. She assumed that the condition was often caused by the aspiration of milk. Parish *et al.* (1960) produced lesions of similar kind in the lungs of guinea pigs by first sensitizing them to cow's milk, and then giving them small amounts of cow's milk via the trachea. Having also found antibodies to different milk proteins in children raised on cow's milk, they concluded that the lesions might be an allergic reaction in the lung between aspirated cow's milk and antibodies to heterologous milk proteins. Most of the subjects in the present study with macrophages of this kind, however, were raised on homologous milk.

Hamperl (1957) and Ricken (1958) described a special sort of pulmonary lesion which they called desquamative pneumonia, but no lesions of their description were found in the present series.

As seen from table 4 and 5, the frequency of macrophages in the alveoli rose with the length of survival of the infant.

Interstitial inflammation

The following conditions were classed as interstitial inflammation in the lung.

(1) Vasculitis and perivasculitis affecting the capillary blood vessels in the interstitial septa (fig. 5). These changes were often of mild character, and often only discovered on careful examination of the slides.

(2) Bronchitis, i.e., diffuse infiltration of polymorphonuclear leukocytes around or in the walls of the bronchi, often accompanied by distinct cellular infil-

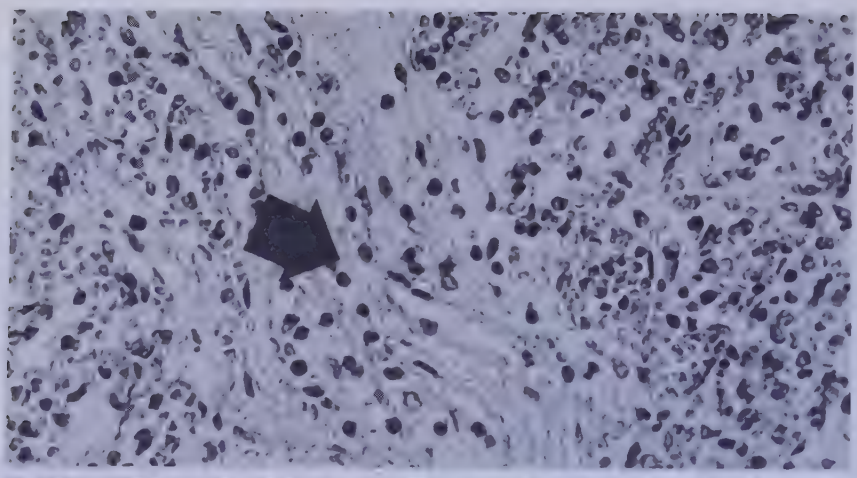

Fig. 5a

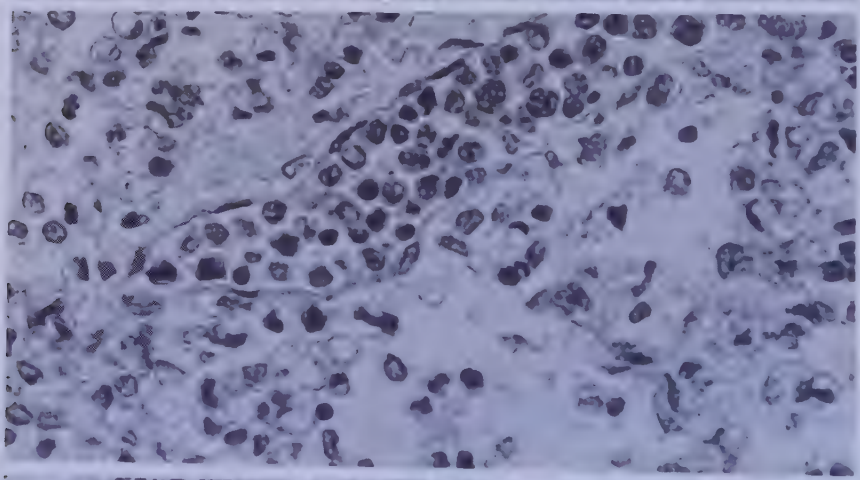

Fig. 5b

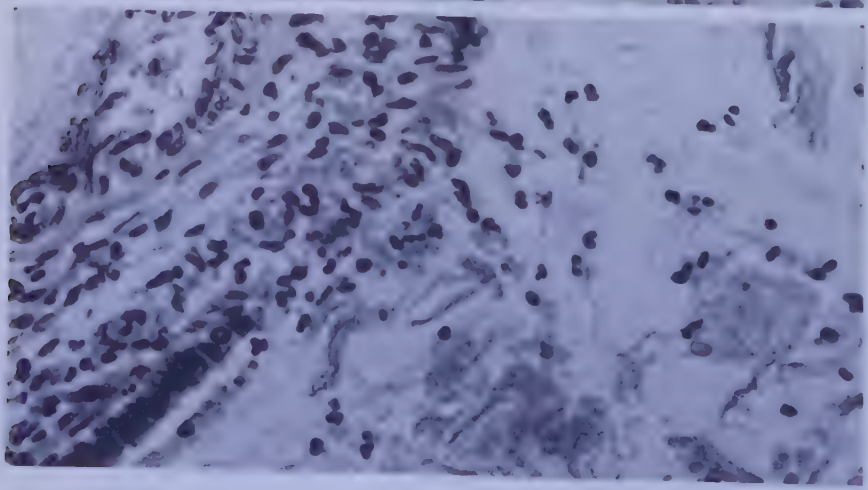

Fig. 6

Fig. 7

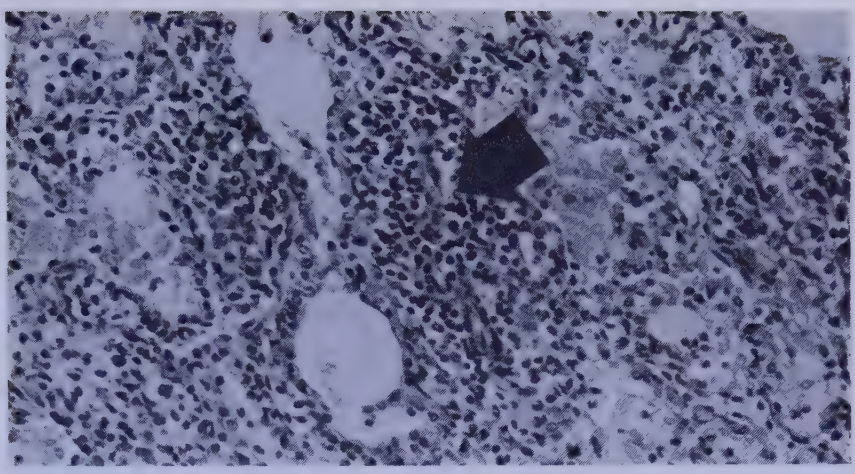

Fig. 5. Interstitial vasculitis in lung. (Pneumococcic infection; case 5, table 26) (a) Perivasculitis in interlobular septum (arrow). Van Gieson, × 250. (b) Accumulation of leukocytes in small vessel in interalveolar septum. H & E., × 450.

Fig. 6. Bronchitis. Perivasculitis affecting small vessels in bronchial wall, and migration of leukocytes into bronchial lumen. Massive aspiration of meconium. Necrotic areas in bronchial epithelium. Lung cultures negative for bacteria. (Liveborn, 3,600 g) PAS, × 300.

Fig. 7. Interstitial inflammation with accumulation of mononuclear cells around small bronchus and alveolar duct. Aspiration of vernix squames. Lung cultures negative for bacteria. (Liveborn, 730 g) H & E., ×200.

tration around the capillary blood vessels in the bronchial walls (fig. 6).

(3) Small clumps of leukocytes, mostly mononuclear, around the bronchioles or alveolar ducts (fig. 7). These were most commonly observed in very immature infants weighing less than 1,000 g, but they were also seen in some term infants.

(4) Granulomas around the bronchi (fig. 11). These were observed in 2 cases of listeriosis, where they were of the same type as the granulomas occurring in other organs in this infection.

Sorba (1948) and Barter (1953) made a special study of interstitial inflammation. Sorba maintained that one could tell whether leukocytes in the alveoli were caused by "true" pneumonia or by the aspiration of amniotic exudate —that if it were "true" pneumonia one could see leukopedesis from the capillary blood vessels, otherwise not. Blanc (1959) used the same criteria as Sorba for "true" pneumonia. Barter (1953) also observed small interstitial infiltrates of mononuclear cells in cases of congenital pneumonia and prenatal aspiration of amniotic fluid, and he produced them experimentally in rabbit fetuses by infecting them with bacteria.

Many of the present subjects showed more than one type of interstitial inflammation, and the first three were pooled for study of the correlation between interstitial inflammation and other types of pulmonary lesion, placentitis and the bacterial findings.

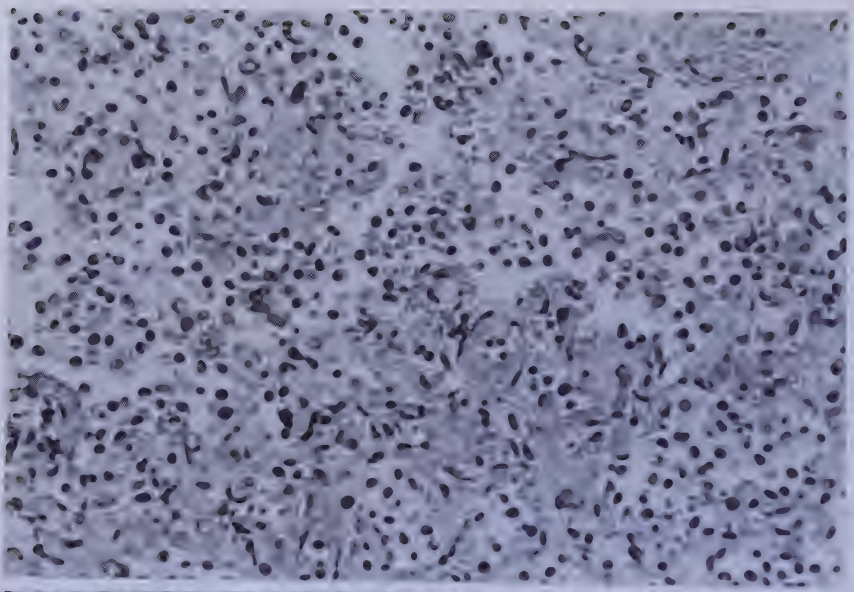

Fig. 8

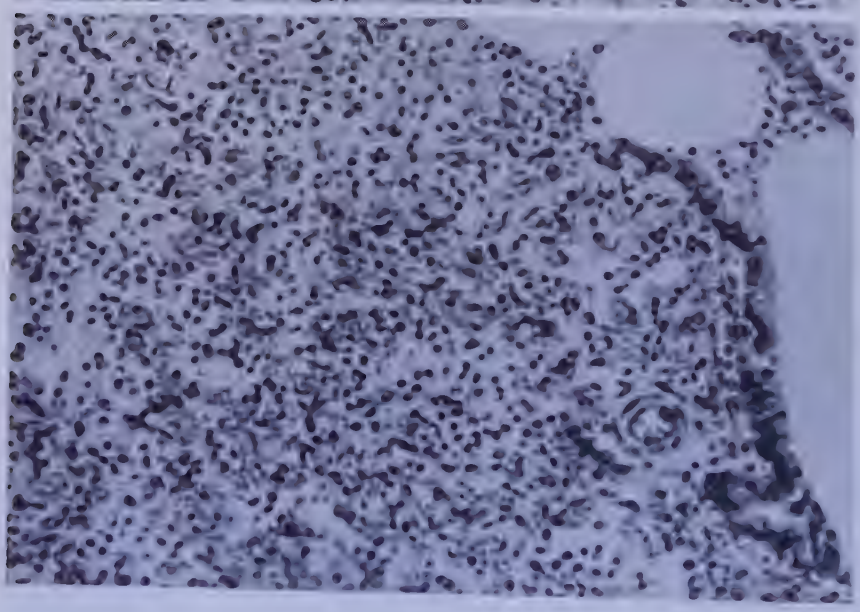

Fig. 9

Fig. 8. Diffuse pneumonia. Infection with coliforms and Staph. aureus. (Stillborn, slightly macerated; case 2, table 26) Van Gieson, × 200.

Fig. 9. Diffuse pneumonia. Pneumococcic infection. (Liveborn; case 3, table 26) Van Gieson, × 200.

Fig. 10

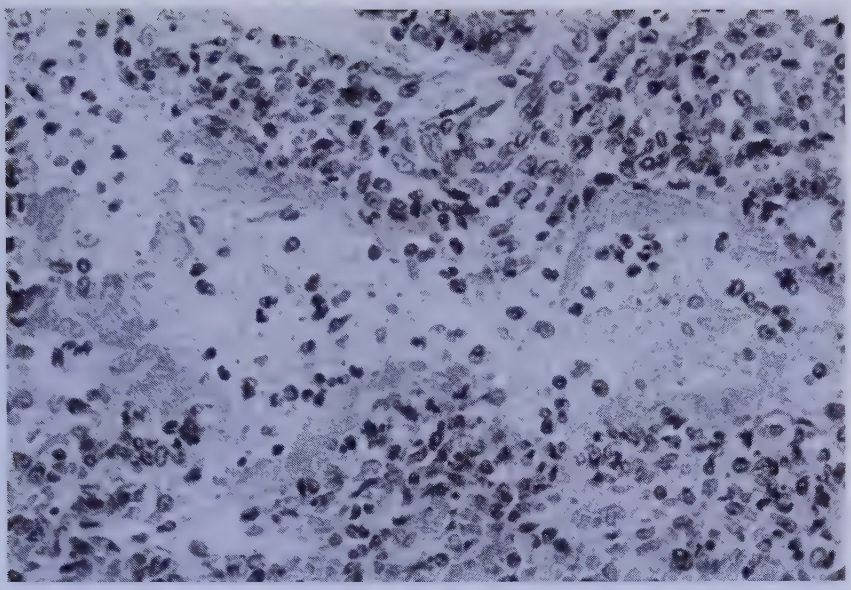

Fig. 10. Detail from lung which showed patchy pneumonia in occasional alveolar ducts combined with hyaline membranes. Lung cultures negative (Liveborn, 1,400 g) H & E., × 300.

Pneumonia

By pneumonia in the present study is meant clumps of polymorphonuclear leukocytes in the alveolar ducts or alveoli. The amount of these leukocytes differed from case to case.

Diffuse (fig. 2, 8, 9). The pneumonia was classed as diffuse when the leukocytes were spread out diffusely over the whole or the major part of the lungs. The leukocytes were generally profuse in these cases, but sometimes differed in number from section to section.

Patchy (fig. 10). The pneumonia was classed as patchy when there were only clumps of leukocytes in occasional alveolar ducts and alveoli. In the stillborn and neonatally dying infants, the leukocytes sometimes showed degenerative changes, including karyorrhexis.

The distinction between diffuse and patchy pneumonia was only made in the cases of stillbirth and death in the first three days of life. In the infants dying later, the pneumonia was of the patchy variety, but the patches sometimes merged with one another into large areas involving the larger part of the pulmonary lobes.

Hemorrhagic. The term hemorrhagic pneumonia was used for diffuse intra-alveolar hemorrhage (massive hemorrhage) combined with intra-alveolar pneumonia.

Granulomatous (fig. 11). Granulomatous pneumonia occurred in 2 cases of listeriosis. These cases had granulomas in the bronchial walls and interstitial septa, and a profuse exudate in the lumina of the bronchi and alveoli.

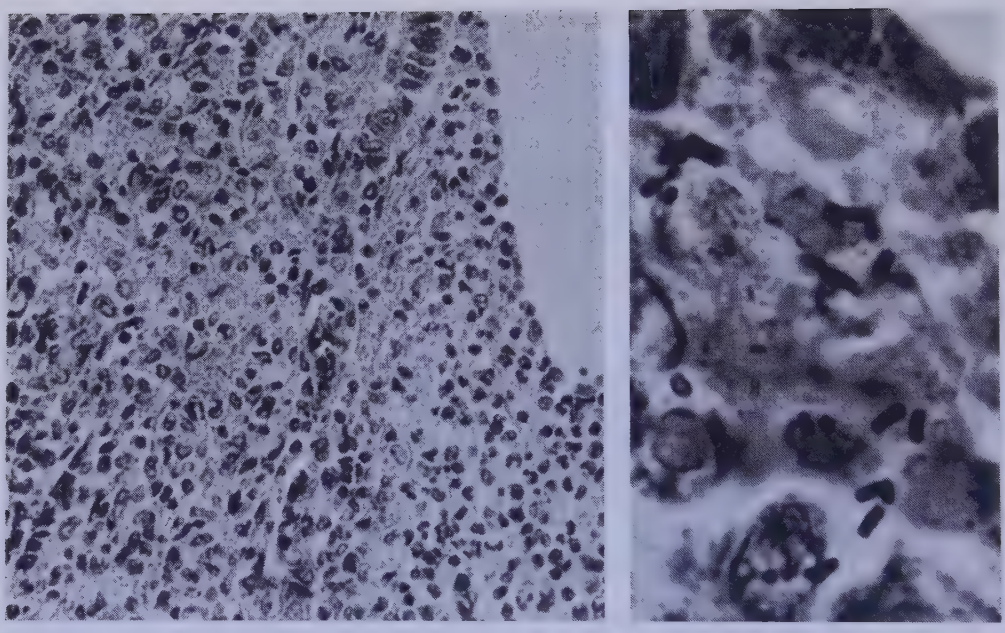

Fig. 11. Granulomatous pneumonia in listeric infection. (Liveborn; case 10, table 26) (a)Granulomatous tissue consisting of mononuclear leukocytes and histiocytes in wall of middle-sized bronchus, and exudate in lumen. H & E. × 300 (b) Listeria rods in the bronchial wall. Levaditi, × 1,800.

Septic infarction (fig. 12). Septic infarcts combined with pneumonia were observed in one case of infection with Staph. aureus. Histological sections in this case revealed thrombi in the vessels and an abundance of gram-positive cocci. Macgregor (1939) described this type of lesion in neonatal infants.

Table 4 and 5 give the frequency of the various types of pneumonia in different groups of subjects.

Lung hemorrhage

Lung hemorrhages were classified according to the method of Potter, as follows.

Petechial hemorrhages in the pleura and septa. Occasional small petechiae were observed both grossly and microscopically under the pleura and in the septa in several cases in the main series: in 35 out of the 72 nonmacerated or only slightly macerated stillborn, in 56 out of the 101 dying during the first three days of life, and in 11 out of the 40 dying between the 4th and 28th day. This common type of hemorrhage is not taken up in the tables in this chapter, and no attempt was made to compare its occurrence with the bacteriological findings.

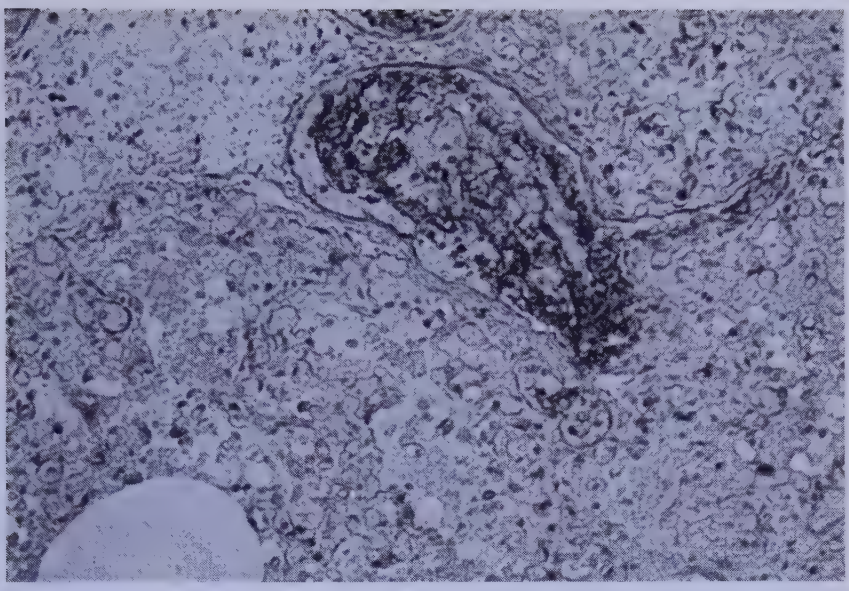

Fig. 12. Septic lung infarction in case of generalized infection with Staph. aureus. The figure shows a thrombus in a middle-sized pulmonary vessel. (Case 17, table 26) PTAH, × 200.

Extensive interstitial hemorrhage (fig. 13). In some cases large hemorrhages were seen around the large vessels near the hilus, as well as diffuse interstitial hemorrhage in the septa. These interstitial hemorrhages contrasted sharply with the air spaces, which were often empty or contained only a few erythrocytes.

Diffuse intra-alveolar hemorrhage (fig. 14). In the cases classed as diffuse intra-alveolar hemorrhage, the air spaces were filled with blood, and the whole or most of the pulmonary lobe was involved. There the blood-filled air spaces contrasted sharply with the interstitial septa, which usually contained no blood or only a few petechiae.

The cases in which there were only occasional small hemorrhages in the alveoli (11 stillborn and 15 liveborn) were not taken up in the analyses of correlations or tables.

As seen from tables 4 and 5, the autopsied Uppsala-born infants differed little from the main series in frequencies of various lesions in the lungs.

Placenta, fetal membranes and umbilical cord

All the anatomical structures in the placenta, fetal membranes and umbilical cord were systematically studied, but

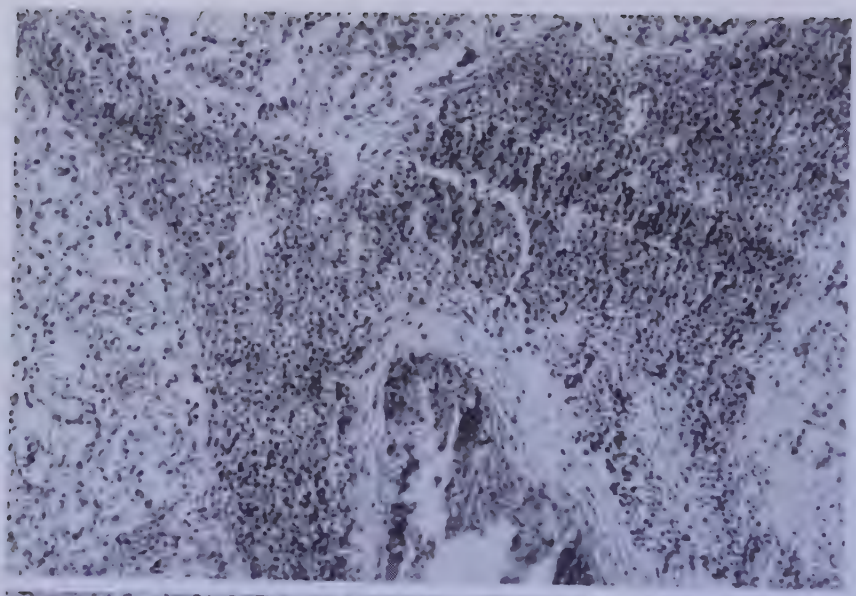

Fig. 13

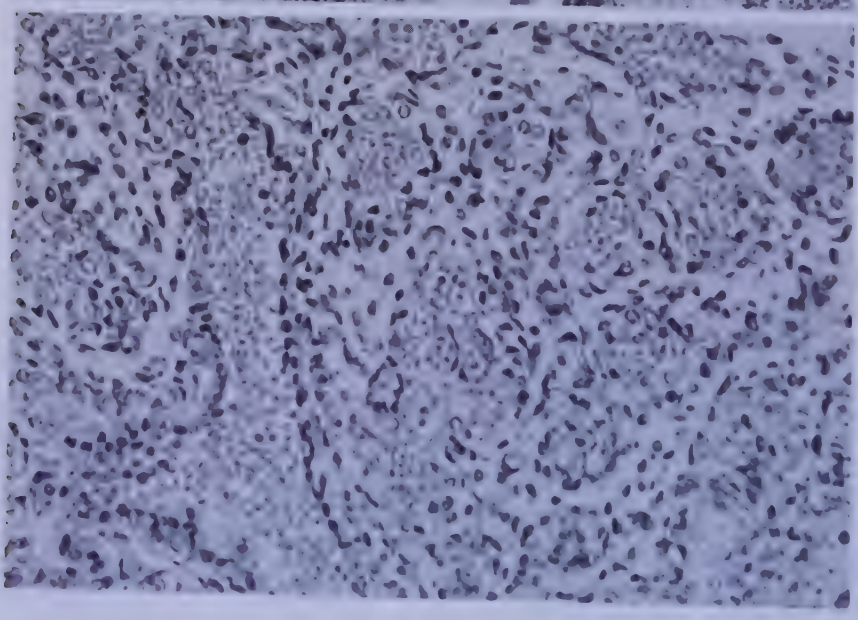

Fig. 14

Fig. 13. Extensive interstitial hemorrhage in lung; large hemorrhage around blood vessel near hilus. Lung culture negative. (Stillborn, 2,980 g) H & E., × 120.

Fig. 14. Diffuse intra-alveolar hemorrhage. No pneumonia. (Stillborn infant infected with beta-hemolytic streptococci, weighing 600 g at birth) H &., × 200.

here I shall only take up the different types of inflammation encountered in these tissues. The types of inflammation occurring there are well known from previous research, and the observations I made correspond in essentials with those made by other authors.

Subchorionic intervillositis and mild chorionitis (fig. 15). Subchorionic intervillositis is characterized by an accumulation of polymorphonuclear leukocytes under and inside the subchorionic layer of the trophoblast in the placenta, which during the second half of gestation is replaced by a fibrinoid substance. The leukocytes are generally assembled in layers and are most numerous at the margin of the placenta.

Subchorionic deciduitis and mild chorionitis. Subchorionic deciduitis and mild

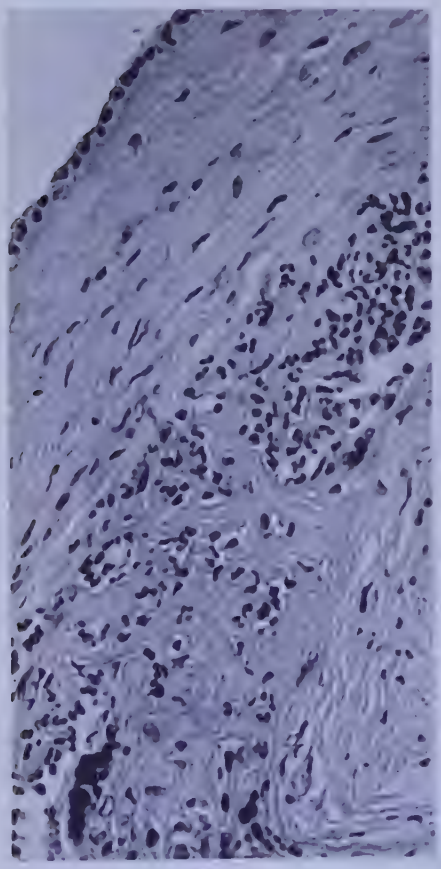

Fig. 15 *Fig. 16*

Fig. 15. Subchorionic intervillositis and mild chorionitis in the placental membranes (incipient placentitis). Note accumulation of leukocytes under and inside the fibrinoid layer of the chorion. H & E., × 150.

Fig. 16. Chorio-amnionitis in placental membranes (full-blown placentitis). Accumulation of leukocytes in the subchorionic fibrinoid layer, and profuse infiltration of cells in chorion and amnion. H & E., × 150.

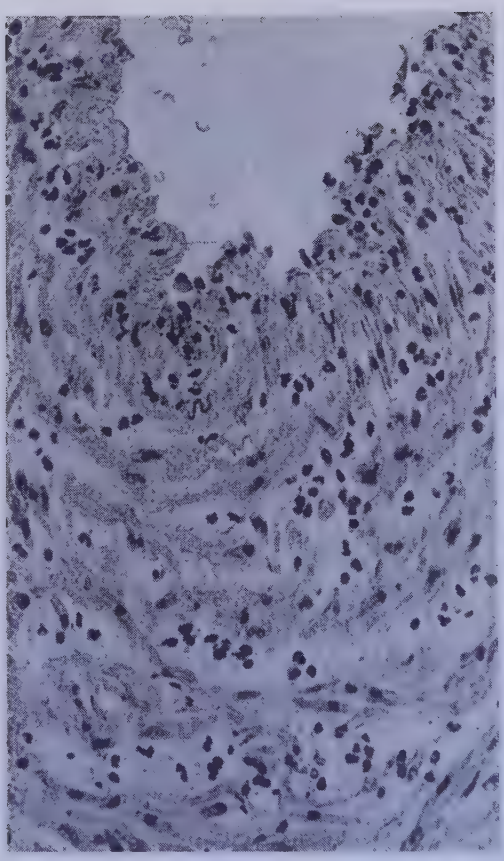

Fig. 17

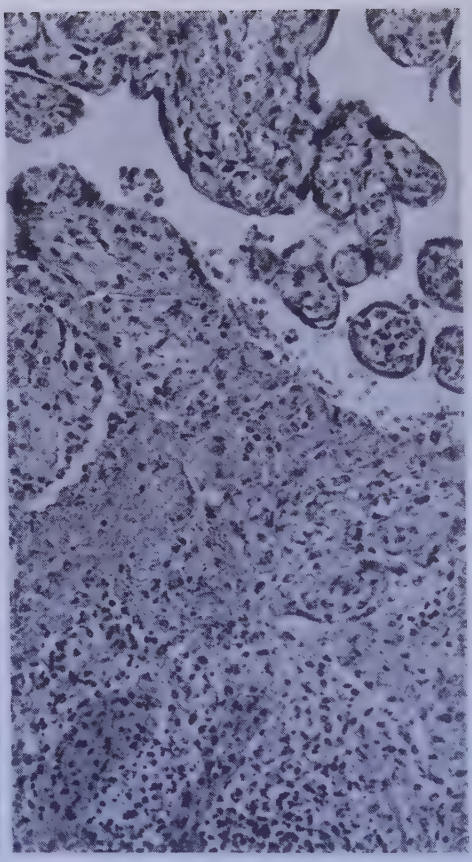

Fig. 18

Fig. 17. Vasculitis in umbilical vein. H & E., × 250.

Fig. 18. Villositis in placenta from greatly macerated stillborn infant with listeric infection. Infarct-like area in the villous part of the placenta with necroses in villi, and profuse infiltration of leukocytes. (Listeria rods were also found in the placenta.) Van Gieson, × 120.

chorionitis is characterized by infiltration of polymorphonuclear leukocytes into the decidua capsularis and the trophoblastic layer of the extraplacental, or reflected, membranes. The inflammation is often patchy, and the decidual and trophoblast cells in the infiltrates are often necrotic. As Küchens (1938) pointed out, most placentas show small perivascular infiltrates of lymphocytes in the decidua. This is probably normal, and should not be confused with the deciduitis associated with placentitis.

Chorio-amnionitis (fig. 16). The term chorio-amnionitis is used for inflammation of the chorion and amnion in the placenta and reflected membranes. It is generally most intense in the subchorionic fibrinoid layer, which often contains dense layers or clumps of polymorpho-

nuclear leukocytes. When it occurs in the membranes covering the placenta, it usually has both a maternal component, caused by leukocytes migrating from the intervillous space up into the stroma of the membranes, and a fetal component, consisting of inflammation in and around the fetal vessels in the chorionic membrane. When it occurs in the reflected membranes, it is probably only a maternal response, originating from the vessels in the decidua capsularis.

Umbilical vasculitis (fig. 17). Umbilical vasculitis develops in the following sequence: margination of leukocytes, migration of the cells into the walls of the vessels, and accumulation of the cells around the vessel walls. The perivasculitis is seen in the shape of sectors with their convexity directed toward the nearest part of the cord surface. (The same is true of the vasculitis in the fetal vessels in the chorionic membranes of the placenta.) The vasculitis is usually most intense in the umbilical vein.

Umbilical vasculitis seems to be usually restricted to the amniotic sac. Thus examination of sections of the umbilical vein in 10 of the present cases of intense umbilical vasculitis showed no inflammation of this vein in the liver.

Villositis (fig. 18). Villositis is characterized by abscesses in groups of chorionic villi. The abscesses contain large quantities of fibrinoid substance, and the

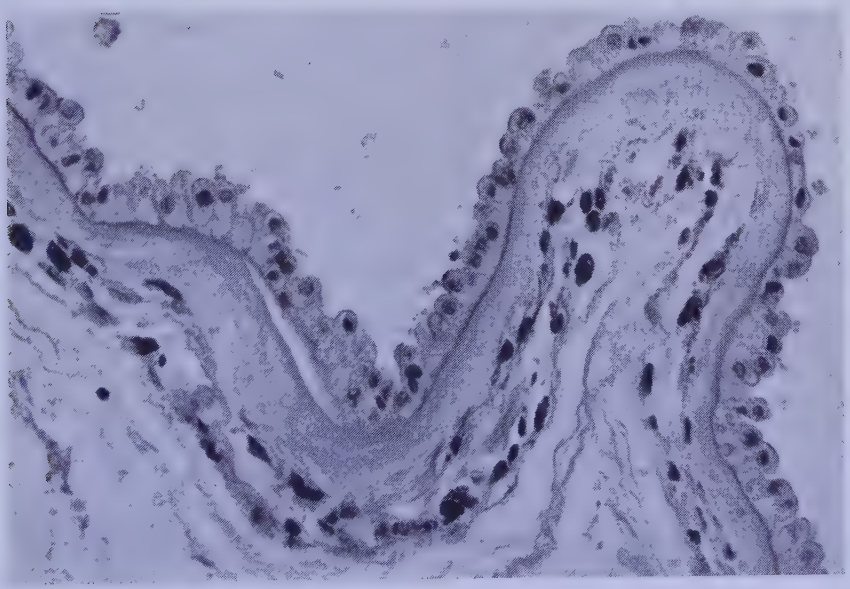

Fig. 19. Macrophages in the amniotic membrane of the placenta, the macrophages containing particles of meconium. PAS, × 250.

trophoblastic layer of the chorionic villi is degenerated. This type of inflammation looks much like an ordinary placental infarct but, unlike an infarct, it is accompanied by widespread chorioamnionitis and umbilical vasculitis. In the present series this type of villositis was only seen in cases of listeriosis.

Inflammation of the decidua basalis. In the present study deciduitis in the margin of the placenta was only found in combination with subchorionic intervillositis, subchorionic deciduitis, or chorio-amnionitis.

Macrophages in the fetal membranes (fig. 19). Macrophages of indistinct outline and variable shape, generally with a weakly eosinophilic cytoplasm, sometimes occur in the fetal membranes. They are a common sequel of intrauterine voiding of the meconium; in the present series the cytoplasm of the cells often contained particles of meconium. Cells of this kind were also seen in some cases of intrauterine hemorrhage, and then contained hemosiderin.

As already pointed out by Blanc (1959), Bourne (1962) and others, and as borne out in this study, macrophages of this kind are common in chorioamnionitis. As seen from table 6, however, they may be seen when there is no inflammation of this kind. It is not certain that macrophages in the membranes are always followed by infiltration with polymorphonuclear leukocytes. For this reason, whenever a placenta only showed macrophages in the membranes, it was not included among the cases of placentitis in this study.

Placentitis. The term placentitis is used in the present study to cover all types and degrees of inflammation in the chorionic and amniotic membranes in the placenta or in the extraplacental membranes, and in the vessels in the umbilical cord.

The term "full-blown placentitis" is used for chorio-amnionitis in the placental part of the membranes. In all except two cases, chorio-amnionitis in the placenta was combined with chorio-

TABLE 6. *Morphological lesions observed in placentas of 111 stillborn infants, grouped by degree of maceration, and of 56 liveborn dying within first three days of life*

Type of morphological lesion in placenta	Nonmacerated or slightly macerated stillborn N=66	Greatly macerated stillborn N=45	Liveborn N=56	Total N=167
Placentitis[a]	33 (8)	28 (21)	23 (7)	84 (36)
Only macrophages in fetal membranes	11	7	11	29
No signs of inflammation	22	10	22	54

[a] Figures in brackets denote number in which placentitis was incipient

amnionitis in the reflected membranes as well, and with but few exceptions also with umbilical vasculitis.

The term "incipient placentitis" is used when there was only subchorionic intervillositis and/or subchorionic deciduitis, together with mild chorionitis, and also when, as rarely, chorio-amnionitis was only seen in the reflected membranes, not in the placental part of the membranes. In all but a few of the cases the intervillositis was situated on the margin of the placenta.

Table 6 shows the number of cases of placentitis, incipient placentitis and only macrophages in the membranes in the dead infants. Placentitis was found in altogether 50 per cent of the dead infants—in 50 per cent of the non-macerated or only slightly macerated stillborn, in 62 per cent of the greatly macerated stillborn, and in 41 per cent of the liveborn dying within three days. When incipient placentitis was excluded, the figures for the four groups dropped to 29, 37, 16 and 28 per cent, and for all the dead infants except the greatly macerated stillborn it was 34 per cent.

In the series of surviving infants (not taken up in table 6), placentitis occurred at a rate of 14 out of 46, or 30 per cent. When the cases of incipient placentitis were excluded, the rate was 7 out of 46, or 15 per cent. The latter percentage does not differ significantly from the corresponding percentage for all the dead infants (29 per cent). When the greatly macerated are excluded from the dead infants, however, a weakly significant difference ($P < 0.05$) emerges between them (34 per cent) and the surviving infants (15 per cent).

Correlation between various morphological lesions in the lung and placenta

Analyses were made of the correlations between the aforedescribed morphological lesions in the lungs, and between them and inflammation in the placenta. Only the cultured nonmacerated or slightly macerated stillborn and liveborn dying within three days (from the main series) were used for these analyses.

No attention was paid to bacteria in this connection.

The 2 cases of the granulomatous variety of pneumonia were excluded from the analyses as this seems to only occur in listeriosis.

Tables 7 and 8 show the number of stillborn and neonatally dying subjects showing different combinations of these morphological lesions.

Significance testing. The correlations were tested for statistical significance with the help of fourfold and sixfold tables.

Fourfold tables were used when the cases were only divided into two classes, for example, into those with and without placentitis, and into those with and without interstitial inflammation in the lung.

Sixfold tables were used whenever the cases were divided into three classes for one lesion, for example, into ones of diffuse, patchy or no pneumonia, and

into ones of massive to moderate, slight, and no prenatal aspiration. Thus, in the sixfold table which follows

	+	(+)	—
+	*a*	*c*	*e*
—	*b*	*d*	*f*

an unbracketed plus sign at the top of the table could stand for massive to moderate prenatal aspiration, a plus sign in brackets for slight prenatal aspiration, and a minus sign for no prenatal aspiration, and the plus and minus signs at the left of the table could stand for presence or absence of pneumonia. After a sixfold table of this kind was completed, it was broken down into three fourfold tables as follows:

	+	(+)—
+	*a*	*c*+*e*
—	*b*	*d*+*f*

	+(+)	—
+	*a*+*c*	*e*
—	*b*+*d*	*f*

	+	—
+	*a*	*e*
—	*b*	*f*

These three tables were then subjected to chi square analysis. The level of significance of the correlations revealed by this analysis is shown in tables 7 and 8. Whenever any of the three fourfold tables differed from the others in the level of significance they yielded, the differing level was given in brackets in tables 7 and 8.

Results. The following were the most important correlations noted:

(1) Pneumonia in the stillborn and liveborn dying within three days was nearly always accompanied by placentitis, judging by the cases in which the placenta was examined.

(2) There was a significant correlation between prenatal aspiration and pneumonia in the liveborn dying shortly after birth, but a weaker and nonsignificant positive correlation in the case of the stillborn. But the two groups of infants differed significantly in this correlation only when cases of prenatal aspiration were divided into ones of massive or moderate aspiration and ones of slight or no aspiration, not when they were divided according to whether or not there were any signs of prenatal aspiration.

(3) There was a weakly significant correlation between signs of prenatal aspiration and placentitis in the liveborn.

To try to determine the connection between prenatal aspiration and pneumonia irrespective of placentitis, the correlation between these two lesions was studied in the 23 cases of stillbirth and 23 of death in the first three days in which placentitis was noted. This revealed a slight positive, but nonsignificant correlation between prenatal aspiration and pneumonia. These results do not appear in the tables.

TABLE 7. *Number of cases with different combinations of pulmonary lesions among the 72 nonmacerated and slightly macerated stillborn and 101 liveborn dying within three days in the main series*

(For total number of subjects with each of separate lesions, see table 5)
*, ** and *** denote significance at the 5, 1 and 0.1 per cent level, respectively.

	Pneumonia		
	Diffuse or patchy	Only diffuse	Interstitial inflammation
Stillborn, nonmacerated and slightly macerated (N=72)			
Prenatal aspiration			
All degrees	8	1	10
Massive or moderate	5	0	7
Interstitial inflammation	8***	4**(*)	—
Hemorrhage			
Extensive interstitial	3	2	4
Diffuse intra-alveolar	1	0	1
Liveborn, dying within 3 days (N=101)			
Prenatal aspiration			
All degrees	16**	7**	20***
Massive or moderate	10 **(*)	7**	14***
Interstitial inflammation	20***	9***	—
Hemorrhage			
Extensive interstitial	2	1	3
Diffuse intra-alveolar	2	1	0
Hyaline membranes	3*	0	4*

TABLE 8. *Number of cases with and without placentitis among those with different pulmonary lesions. Cases in main series of nonmacerated and slightly macerated stillborn and of death within three days in which placenta was examined*

*, ** and *** denote significance at the 5, 1 and 0.1 per cent level, respectively;
(0) not significant

	Placentitis[a]		No placentitis		Significance of differences in	
Pulmonary lesion	Stillborn (N=33)	Liveborn (N=23)	Stillborn (N=33)	Liveborn (N=33)	Stillborn	Liveborn
Prenatal aspiration						
All degrees	18	12	18	6	(0)	*
Massive or moderate	15	7	11	2	(0)	*
Interstitial inflammation	12	12	4	2	*	***
Pneumonia						
Either diffuse or patchy	8	13	1	1	*	***
Only diffuse	4	4	0	0	(0)	(0)

[a] including incipient stages

(4) There was a highly significant correlation in both the stillborn and liveborn between interstitial inflammation and pneumonia, and in the liveborn between interstitial inflammation and prenatal aspiration, and there was a weakly significant correlation in the stillborn and a highly significant correlation in the liveborn between interstitial inflammation and placentitis.

Discussion

It is interesting to see the frequencies of the different lesions of the lungs in the present study, and how they compare with those other authors have observed. For this comparison, it is most suitable to use the consecutive series of all Uppsala-born infants (table 4).

The literature does not seem to contain any specific information on the frequency of signs of intense prenatal aspiration of particulate matter. Morison (1963) said that appreciable amounts of such material were found in about 10 per cent of stillborn. In the Uppsala infants, signs of intense aspiration (classed as massive or moderate) occurred in 35 per cent of the stillborn (table 4).

Potter (1961) said that it might be hard to detect signs of prenatal aspiration in very immature infants, because the amniotic fluid often contains no vernix squames at this stage. This possibility does not seem to have affected the figures for prenatal aspiration in the Uppsala-born series to any great extent: 36 out of the 177 stillborn and liveborn dying within three days, i.e., 20 per cent, weighed less than 1,000 g at birth, and 7, or 16 per cent, of the 43 infants showing signs of massive to moderate aspiration weighed less than 1,000 g. As seen, the percentages are much alike. On the other hand, it may have been that some cases of slight prenatal aspiration escaped the morphological examination, for if there were only occasional clumps of vernix squames in the lung they might not have appeared in the sections removed. The figures for slight prenatal aspiration in this series, therefore, are probably minimum figures.

It is hard to compare the frequency of pneumonia in the present study with that in others, as other authors have not always said exactly what they meant by "pneumonia". Pneumonia has been found in 12 to 15 per cent of the stillborn in large autopsy series (see chap. 1). This is in agreement with the 15 per cent frequency found for the stillborn Uppsala infants. For all the Uppsala-liveborn dying within the first 28 days of life, the frequency was 30 per cent. Macgregor (1939), in her extensive analysis of a large autopsy series, found pneumonia in 37 per cent of infants dying during the same time of life. Morison (1963) said that pneumonia caused the death of between 17 and 27 per cent of a large series of autopsied infants weighing over 1,000 g at birth and dying within 28 days. Nineteen per cent of the liveborn Uppsala infants had pneumonia when the ones weighing less than 1,000 g at birth were excluded.

Accordingly, pneumonia occurred at about the same rate in the present study as in the large series described by other authors. Apparently, pneumonia is still as common among the stillborn and

neonatally dead as it was 40 years ago, when Johnson & Meyer made their extensive study of "congenital" pneumonia.

Diffuse intra-alveolar lung hemorrhage was found in about 6 per cent of the liveborn in the Uppsala-born infants (table 4). This agrees with the 6.6 per cent rate Ahvenainen & Call (1952) found in an unselected autopsy series of 802 liveborn dying within the first month of life. In the Uppsala infants, however, this type of hemorrhage was found most often in the liveborn dying between the 4th and 28th day of life, while Ahvenainen & Call found it most often in infants dying between the 1st and 3rd day. This difference is apparently not only owing to their having defined massive hemorrhage differently from the way it was done here. As mentioned, infants with only occasional small intra-alveolar hemorrhages in the lungs were not grouped under diffuse pulmonary hemorrhage. But even when they are included, the frequency of pulmonary hemorrhage remained lower during the first three days of life than during the 4th to 28th day—13 against 39 per cent. The rates for the Uppsala-born infants dying between the 4th and 28th day of life cannot be relied upon too much, however, because of the small number of cases in this group (table 4).

In large series of consecutive deliveries published by Benirschke & Clifford (1959) and Benirschke (1960), chorioamnionitis (here designated as full-blown placentitis) was observed in 12 to 13 per cent of cases. It was noted in 15 per cent of the 46 present cases of surviving infants. This is a weakly significantly ($P < 0.05$) smaller percentage than the 34 per cent observed for the dead infants (excluding the greatly macerated stillborn). It would appear, therefore, that placentitis, at any rate of the full-blown variety, occurs more often in cases of stillbirth or death in the first three days of life than in cases where the infants survive. It is true that the placenta was not examined for 55 of the 177 nonmacerated or slightly macerated stillborn and liveborn dying within three days who were born in Uppsala. Pneumonia was found in 28 per cent of these 55. The corresponding figure for all the 177 infants was 23 per cent. The nonsignificant difference between these percentages, together with the close connection noted between placentitis and pneumonia both in this study and others indicate that the figures from the missing placentas would not have caused much change in the frequency of placentitis for all the 177 dead infants in the consecutive autopsy series of Uppsala-born infants.

Most of the interest attaching to the morphological lesions in the present study lies in how they were related to the bacteriological observations, and this relationship will be discussed in chapter 6.

CHAPTER 5

BACTERIOLOGICAL RESULTS

As seen from table 9, the cases differed slightly in number of organs examined. One reason for this is that at the start of the investigation it was thought that macerated children were not suitable for culture, and so the stillborn had fewer organs examined on the whole than did the liveborn. Another reason is that the liver was not routinely examined at the start of the investigation, and the liver is missing from most of the cases in which five organs were examined. In two cases, only the lung and blood were examined.

The mean number of organs cultured per subject amounted to 4.8 for all the stillborn, to 4.5 for macerated stillborn, to 5.3 for all the liveborn, and to 5.4 for the liveborn dying within three days after birth.

Frequency of positive and negative cultures and analysis of possible sources of error

Uniformly negative cases in different groups of infants

Table 9 also shows the number of infants in which all the organs cultured were negative. They amounted to 71 per cent of all the stillborn, 72 per cent of the macerated stillborn, 46 per cent of all the liveborn, 61 per cent of the liveborn dying within three days after birth, and 58 per cent of the whole series of 264 infants. (For the Uppsala-born infants only, the corresponding percentages were 69, 70, 52, 63, and 61 per cent.)

Percentage of positive cultures in separate organs in different groups of subjects. Comparison between different groups in this respect

Table 10 shows the percentage of positive cultures obtained from the separate organs both in the whole series and in various subgroups.

As before, the stillborn subjects were divided into nonmacerated, slightly macerated and grealty macerated in this table, because only the first two groups can be studied histologically. It is also advisable to group them this way for bacteriological reasons: generally speaking, the degree of maceration depends on the length of time between the intrauterine death and delivery, and the length of this interval may in itself affect the results of culture, apart from its effect on the degree of maceration.

Likewise, the liveborn were divided into ones dying within three days, within

TABLE 9. *Stillborn and liveborn subjects by number of organs included in routine culture studies*

Figures in brackets denote number of infants from whom all cultures were negative

No. of organs studied among: lung, blood, spleen, liver, kidney and intestine	All stillborn	Macerated stillborn	All liveborn	Liveborn dying within 3 days
6	40 (30)	15 (12)	79 (36)	61 (35)
5	37 (23)	18 (9)	39 (16)	23 (14)
4	31 (20)	22 (16)	16 (8)	10 (8)
3	13 (12)	12 (11)	7 (5)	7 (5)
2	2 (2)	1 (1)	0	0
Total	123 (87)	68 (49)	141 (65)	101 (62)
Mean no. of cultures per subject	4.8	4.5	5.3	5.4

the 4th to 7th day, and after the first week of life. The liveborn dying within 24 hours after birth are also taken up separately in this table. As seen there, the percentages of positive cultures in this group were almost the same as those in the combined stillborn. But, as this does not necessarily mean that the bacteria were present before birth in all the cases of death within 24 hours, they were not grouped together with the stillborn in the following.

The Uppsala-born stillborn and liveborn dying within three days gave the same percentages of positive cultures as the corresponding groups in the whole series, or ones at the most 1 per cent lower. The combined liveborn among the Uppsala-born infants gave percentages between 2 and 5 per cent lower than in the whole series, except for the lung for which they had the same percentage of positive cultures.

Table 11 shows the significance of differences between different subject-groups in percentage of positive cultures from separate organs. It is seen from this and the foregoing table that the separate organs yielded much the same results in the stillborn and in the liveborn dying within three days, the only exception being that the intestine was weakly significantly more often positive in the liveborn. The results were also much alike for the macerated and non-macerated stillborn, none of the differences between them reaching a significant level. The tables also show that the frequency of positive cultures from the liveborn rose sharply with the length of survival; this was true for all the organs, but the rise set in earlier and more sharply for cultures of the lung, intestine and kidney.

Comparison between organs within separate groups of subjects for results of culture

It cannot be seen from tables 10 and 11 how the organs differed from one another in frequency of positive cultures within separate groups of subjects, because the infants varied slightly in number of organs cultured.

TABLE 10. *Percentage (± standard error) of positive cultures from separate organs in different groups of infants*

Figures in brackets denote number of cultures of organ in question

	Lung	Blood	Spleen	Liver	Kidney	Intestine
Whole series	30±2.8 (263)	12±2.2 (225)	17±2.4 (240)	14±2.6 (181)	19±2.7 (212)	32±3.1 (225)
All stillborn	21±3.7 (123)	8±2.9 (91)	12±3.2 (106)	9±3.1 (86)	9±3.0 (89)	13±3.4 (97)
Nonmacerated stillborn	22±5.6 (55)	6±3.5 (49)	11±4.3 (54)	8±4.6 (36)	4±2.9 (45)	9±4.2 (47)
Slightly macerated stillborn	29±11.3 (17)	13±9.0 (15)	19±10.1 (16)	10±10.0 (10)	7±6.8 (15)	20±10.7 (15)
Greatly macerated stillborn	18±5.4 (51)	7±5.0 (27)	11±5.3 (36)	10±4.8 (40)	17±7.1 (29)	17±6.4 (35)
All liveborn	37±4.1 (140)	16±3.2 (134)	20±3.5 (134)	18±4.0 (95)	27±4.0 (123)	45±4.4 (128)
Liveborn dying within 24 hours	20±4.5 (80)	7±3.0 (73)	8±3.2 (74)	9±3.8 (57)	8±3.4 (65)	14±4.1 (73)
Liveborn dying within 3 days	26±4.4 (101)	7±2.6 (94)	9±2.9 (96)	10±3.5 (73)	12±3.5 (86)	27±4.6 (93)
Liveborn dying on 4—7th day	71±11.3 (17)	24±10.7 (17)	35±11.9 (17)	30±15.3 (10)	53±12.5 (17)	87±9.0 (15)
Liveborn dying on 8—28th day	64±10.5 (22)	43±10.5 (23)	55±10.9 (22)	58±14.9 (12)	70±10.5 (20)	100 (20)

To enable this comparison, the following calculations were done. Two organs were compared at a time. The individual organs for each two were paired according to different combinations of positive and negative results of culture, and the number of pairs with each combination entered into tables like the following.

All liveborn

		Lung +	Lung —
Blood	+	16	4
	—	35	78

The contrast, or difference between, the number of organs with opposite results of culture was studied, thus in this

TABLE 11. *Differences between pairs of subject groups in percentage positive cultures from separate organs, and standard errors of differences*

*, **, and *** denote significance at the 5, 1 and 0.1 per cent level, respectively

Groups compared (First group minus second)	Positive cultures from Lung		Blood		Spleen		Liver		Kidney		Intestine	
	Diff. in %	SE of diff.	Diff. in %	SE of diff.	Diff. in %	SE of diff.	Diff. in %	SE of diff.	Diff. in %	SE of diff.	Diff. in %	SE of diff.
All liveborn — all stillborn	16**	5.5	8	4.3	8	4.7	9	5.1	18**	5.0	32***	5.6
Nonmacerated — slightly macerated stillborn	—7	12.6	—7	9.6	—8	11.0	—2	11.0	—3	7.4	—11	11.5
Nonmacerated — greatly macerated stillborn	4	7.8	—1	6.1	0	6.8	—2	6.6	—13	7.7	—8	7.7
Slightly — greatly macerated stillborn	11	12.5	6	10.3	8	11.4	0	11.1	—10	9.8	3	12.5
Nonmacerated — all macerated stillborn	1	7.5	—4	5.9	—3	6.5	—2	6.3	—10	6.0	—9	6.9
Liveborn dying within 3 days — ones dying on 4—7th day	—45**	12.1	—17	11.0	—26*	12.2	—20	15.7	—41**	13.0	—60***	10.1
Liveborn dying on 4—7th day — ones dying on 8—28th day	7	15.4	—19	15.0	—20	16.1	—28	21.3	—17	16.3	—13	9.0
Liveborn dying within 3 days — ones dying on 8—28th day	—38**	12.4	—36**	10.8	—46***	11.3	—48**	15.3	—58***	11.1	—73***	4.6
Liveborn dying within 3 days — all stillborn	5	5.7	—1	3.9	—3	4.9	1	4.7	3	4.6	14*	5.7
Liveborn dying within 3 days — nonmacerated stillborn	4	7.1	1	4.3	—2	5.2	2	5.8	8	4.5	18**	6.2
Liveborn dying on 4—7th day — all stillborn	50***	11.9	16	11.0	23	12.3	21	15.6	44***	12.7	74***	9.6

case between 35 and 4, and the exact binomial distribution at a P of .50 was used for analysis of the significance (sign test). Using this method, one can see whether or not the difference in the number of positive cultures yielded by the two organs was due to chance. The level of significance was denoted as 0.05, 0.01 and 0.001.

Table 12 gives the results of these analyses. To save space, only the level of significance of the differences is set out there. The liveborn were divided into ones dying after the 3rd day and ones dying within three days. The dividing line was put at three days because the liveborn dying before this time were more like the stillborn in the outcome of different organ cultures than were the liveborn dying later (table 10). All the stillborn were collected in one group, because they gave too few positive cultures to allow analysis of subgroups. As seen from the table, some organs were not compared for the stillborn. This was because there were not more than five positive examples of each of the two organs in question in this group of subjects.

TABLE 12. *Significance of differences between various pairs of organs in number of positive cultures, in different groups of subjects*

Figures in brackets show number of pairs compared. *, **, and *** denote significance at the 5, 1 and 0.1 per cent level, respectively; (0), not significant, (a) significance not tested because neither organ yielded more than 5 positive cultures

Organs compared (The first in each pair had the largest number of positive cultures)	Significance of difference between compared organs in number of positive cultures			
	All stillborn	All liveborn	Liveborn dying within 3 days	Liveborn dying after 3rd day
Lung — blood	*** (91)	*** (133)	*** (94)	** (39)
Lung — spleen	** (106)	*** (134)	*** (96)	(0) (38)
Lung — kidney	** (89)	** (118)	** (82)	(0) (36)
Lung — liver	* (87)	*** (94)	** (73)	** (21)
Spleen — blood	(0) (86)	(0) (130)	(0) (91)	(0) (39)
Liver — blood	(a) (58)	(0) (89)	(0) (67)	(0) (22)
Kidney — blood	(0) (71)	* (120)	(0) (83)	* (37)
Spleen — liver	(0) (71)	(0) (89)	(0) (68)	(0) (21)
Kidney — spleen	(0) (82)	* (122)	(0) (69)	(0) (37)
Kidney — liver	(0) (61)	(0) (85)	(0) (66)	(0) (19)
Intestine — blood	(a) (72)	*** (128)	*** (93)	*** (35)
Intestine — spleen	(0) (86)	*** (123)	*** (89)	*** (34)
Intestine — liver	(0) (67)	*** (89)	* (68)	** (21)
Intestine — kidney	(a) (75)	*** (115)	** (82)	*** (33)

The following are the most important observations in table 12.

(1) In the combined stillborn and combined liveborn the lung was significantly or highly significantly more often positive than the blood. The same was true of the lung compared with the spleen, liver and kidney in the stillborn, the combined liveborn, and the liveborn dying within three days.

(2) In the combined liveborn, the intestine was significantly or highly significantly more often positive than the other abdominal organs or blood.

(3) In the combined liveborn, the kidney was weakly significantly more often positive than the blood and spleen ($P < 0.05$).

One cannot draw any definite conclusions from the fact that the blood, spleen and liver did not differ significantly from each other, because the number of cases with deviating results (for example, 4 + 35 in the foregoing fourfold table) were always less than 25.

TABLE 13. *Cases receiving antibiotics and/or chemotherapeutics by type of drug given*

Drugs given	Stillborn[a]	Liveborn dying within 3 days of birth			Liveborn dying after 3rd day
		Mother	Child	Both	
Penicillin	11	11	—	—	—
Sulfonamides	1	4	—	—	—
Penicillin and sulfonamides	5	—	—	—	—
Tetracyclines	—	—	28	—	25
Combination of drugs	1[c]	—	—	2[b]	4
Total	18		35		29
Total cases examined	123		101		40

[a] Mothers treated during or a few days before delivery
[b] Penicillin and sulfonamides to mother, tetracyclines to child
[c] Penicillin and tetracyclines

TABLE 14. *Percentage of positive cultures from separate organs in cases given and not given antibiotics and/or chemotherapeutics*

Organ	Stillborn				Liveborn dying within 3 days				Liveborn dying after 3rd day			
	Treated		Untreated		Treated		Untreated		Treated		Untreated	
	No. cult'd	% pos.	No. cult'd	% pos.	No. cult'd	% pos.	No. cult'd	% pos.	No. cult'd	% pos.	No. cult'd	% pos.
Lung	18	28	105	20	35	23	66	27	29	72	10	50
Blood	15	7	76	8	32	0	62	11	29	28	11	55
Spleen	17	12	89	12	34	12	62	8	28	39	11	64
Liver	10	20	76	8	25	8	48	10	17	47	5	40
Kidney	12	8	77	9	31	10	55	13	26	62	11	64
Intestine	14	21	83	12	30	37	63	22	25	92	10	100

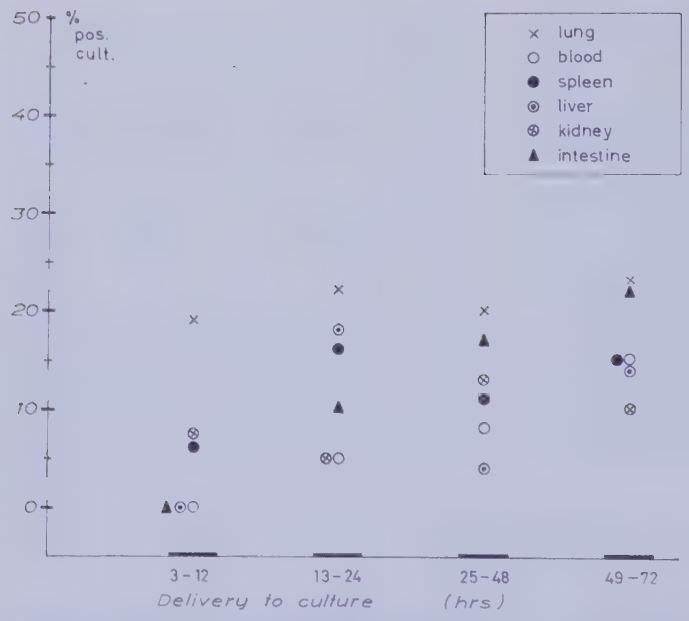

Fig. 20. Percentage of positive cultures obtained from various organs of the stillborn, divided by different lengths of time between delivery and autopsy.

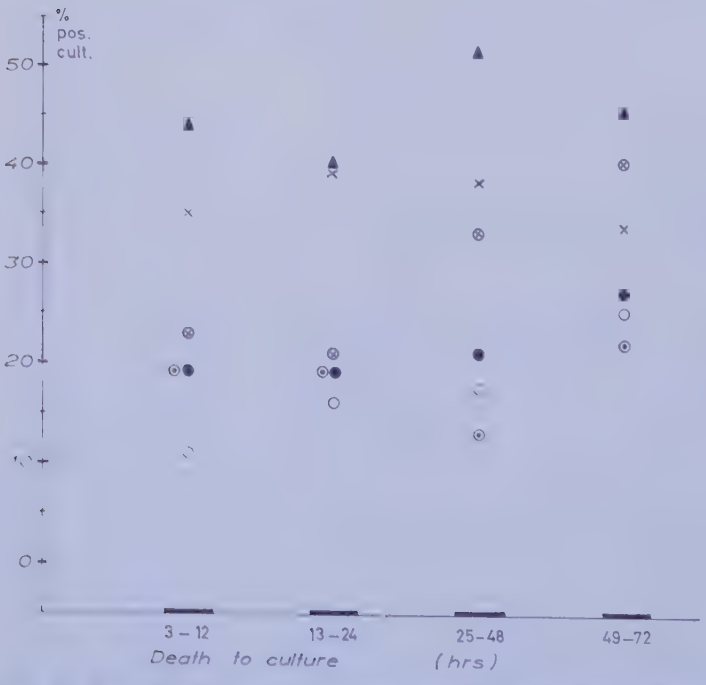

Fig. 21. Percentage of positive cultures obtained from various organs of the liveborn, divided by different lengths of time between death and autopsy. For meaning of symbols, see figure 20.

Effect on results of culture of previous treatment with antibiotics and chemotherapeutics

Table 13 gives the number of cases in various groups of infants in which antibiotics and chemotherapeutics were given, and what drugs were used. For the stillborn, the number of mothers treated during or for a few days before delivery is set out. For the liveborn dying within three days, the number of cases in which the mother, the child or both, were given these drugs is shown.

As Charles (1954) demonstrated, penicillin, terramycin and the like can easily pass from mother to fetus to reach a therapeutic concentration in the fetus. Essentially the same is true of different kinds of sulfonamides (Held & Egger, 1946; von Friesen, 1951; Sparr, 1958).

Table 14 gives the percentage of positive cultures from the separate organs in the treated compared with the untreated cases. No consideration is given in this table to the type or dose of the drug, to the duration of treatment, or to the type of bacteria found.

As seen there, the blood was positive in 11 per cent of the untreated liveborn dying within three days, as against none of the treated, the difference being 11 per cent. The corresponding figures for the liveborn dying after the 3rd day were 55 and 28 per cent, the difference being 27 per cent. Standard weighting of these two differences yields a significant difference ($P < 0.05$) if both groups of liveborn are weighted together. No significant difference emerged between the untreated and treated groups as regards percentage of positive cultures from the other organs.

Effect on results of culture of interval between death or stillbirth and autopsy

In figures 20 and 21, the stillborn are divided according to the length of time between delivery and autopsy and the liveborn according to length of time between death and autopsy. It was not hard to determine the length of these intervals. The shortest interval amounted to three hours.

Table 15 shows the differences between the different interval groups in frequency of positive cultures and the standard error of their differences. As seen, the stillborn autopsied 3—12 hours after delivery differed weakly significantly ($P < 0.05$) in percentage of positive cultures from the intestine from the stillborn autopsied within 25—48 hours. Nowhere else was there a significant difference. Separate analysis of the liveborn dying within three days after birth (not taken up in the table) gave the same statistical results as in the stillborn.

To see whether the length of this interval had a significant effect on the results of culture on the whole, the following calculation was done: Each infant was assigned an index, consisting of the number of positive cultures it gave divided by the total number of cultures done in its case, converted to percentages between 0 and 100. To get figures for the groups, the index values for the individuals in the group were added, and the mean index ($\bar{x}$), standard deviation (SD) and standard error (SE) calculated. The following is an example.

All liveborn, 3—12 hours between death and autopsy

Positive cultures/ total cultures per case	Index value (%), viz. 100×foregoing quotient	No. of cases	Index value × no. of cases
0/6	0	13	0 × 13 = 0
1/6	17	4	17 × 4 = 68
2/6	33	3	33 × 3 = 99
3/6	50	1	50 × 1 = 50
4/6	67	2	67 × 2 = 134
5/6	83	1	83 × 1 = 83
6/6	100	2	100 × 2 = 200
0/5	0	8	0 × 8 = 0
1/5	20	4	20 × 4 = 80
etc.			
N (total number of cases):		49	
			Total 1,264

$\bar{x}$: $\frac{1,264}{49} = 25.8$ SD: 33.5 SE: 4.8

Table 16 shows the results of calculating these indices for all the stillborn, all the liveborn, and the liveborn dying within three days.

Analysis reveals that there was no significant difference between different lapses of time in either group of liveborn. But the stillborn autopsied within 12 hours after delivery differed weakly significantly ($P < 0.05$) from the ones autopsied within 13—72 and within 25—72 hours after delivery.

As it was, however, the proportion of nonmacerated, slightly macerated and greatly macerated stillborn differed with different lengths of time between delivery and autopsy. After standard weighting was done to keep the proportions the same when the series was divided according to different intervals, the significant difference between the 3—12 hour group and the groups with other intervals disappeared.

Cultures of placentas and fetal membranes

As was seen from table 17, the different groups of the dead infants did not differ significantly in frequency of positive cultures either from the placenta or from the fetal membranes. The table also showed that the fetal membranes were much more often positive than the placentas. This is probably because the membranes are often contaminated by the flora in the mother's vagina or rectum during their expulsion. (It was not possible to sterilize the surface of the fetal membranes the same way as that of the placenta and fetal organs.)

As for the 46 surviving infants (not shown in table 17), the placentas were positive in 8 cases, or 17 per cent, and the fetal membranes in 29 cases, or 63 per cent.

These percentages for the surviving cases did not differ significantly from

TABLE 15. *Difference between different lapses of time between stillbirth or death and autopsy in percentage of positive cultures obtained from various organs*

*, **, and *** denote significance at the 5, 1 and 0.1 per cent level, respectively

	Percentage of positive cultures in cases of first interval minus ones of second interval							
	Stillborn				Liveborn			
Organ	25—48 hr. minus 3—12 hr. cases %	SE of diff.	49—72 hr. minus 3—12 hr. cases %	SE of diff.	25—48 hr. minus 3—12 hr. cases %	SE of diff.	49—72 hr. minus 3—12 hr. cases %	SE of diff.
Lung	1	10.9	4	11.3	3	5.1	—2	15.8
Blood	8	4.6	15	8.2	7	7.8	14	13.9
Spleen	5	8.0	9	9.3	2	8.8	8	15.1
Liver	4	4.0	14	6.7	—6	10.0	3	16.3
Kidney	6	9.1	3	9.5	10	10.1	17	17.5
Intestine	17*	6.3	22	8.2	7	11.1	1	17.5

those for the liveborn dying within three days (34 and 59 per cent). Nor were there any significant differences between the surviving cases, on the one hand, and a combination of the liveborn dying within three days and the nonmacerated and slightly macerated stillborn, on the other, the percentage of positive placentas in the combined group amounting to 26 and of positive membranes to 54.

If the word positive is only used for cultures yielding pathogenic bacteria, the placentas were positive in 28 per cent of the cases of liveborn dying within three days and in 9 per cent of the surviving infants. The corresponding figures for the membranes were 37 and 17 per cent, respectively. Though these differences seem large, they were not statistically significant ($P > 0.05$). Nor did the surviving infants differ significantly in these respects from the combined group of liveborn dying within three days and the nonmacerated or slightly macerated stillborn.

Types of bacteria found

Table 18 sets out the different kinds of bacteria found in the fetal organs. All the organs examined routinely are included there: lung, blood, spleen, liver, kidney and intestine. Each type of bacteria is only listed once for each case, regardless of the number of organs from which it was grown in the case, and no consideration is given to whether it occurred alone or together with others.

As seen there, altogether 112 out of the 264 infants yielded bacteria from one or more organs.

The coliform bacteria predominated over the other bacteria in all 112 infants whose organs yielded bacteria, occurring in 63 per cent. Enterococci were found in 24 per cent. Alpha-hemolytic streptococci occurred in only 8 per cent. Streptococci of the beta-hemolytic type were present in only 3 cases, or 3 per cent. Ps. aeruginosa was found in 5 per cent, and pneumococci were only isolated

TABLE 16. *Length of time between stillbirth or neonatal death and autopsy in relation to number of positive cultures obtained from the organs as a whole, expressed by an index*

N, total number of cases of interval in question; $\bar{x}$ mean index of infant group with time interval in question; the computation of the individual index is described on p. 49. SE, standard error of $\bar{x}$.

	Hours between stillbirth or death and autopsy											
	3 — 12			13 — 24			25 — 48			48 — 72		
	N	$\bar{x}$	SE	N	$\bar{x}$	SE	N	$\bar{x}$	SE	N	$\bar{x}$	SE
All stillborn	21	5.4	2.7	27	13.4	4.8	40	13.2	4.2	35	16.9	4.8
All liveborn	49	25.8	4.8	38	26.7	5.7	43	32.7	5.2	11	24.8	12.0
Liveborn dying within 3 days	35	17.5	5.0	26	10.3	4.1	32	23.3	5.3	8	4.1	4.1

from one child (1 per cent). Salmonella (group C) was only found in the intestine of a 27 day old child showing no morphological or clinical signs of infection. Listeria were isolated from 7 cases (6 per cent); in 2 of these they only grew in enrichment media and in only one organ—once in the liver and once in the lung. Staph. aureus was registered in 22 cases, or 20 per cent, and Staph. albus in 27 cases, or 24 per cent, most of them liveborn. Diphtheroid bacteria, lactobacilli, A. fecalis or Proteus were isolated in a few cases. One case showed gram-negative bacteria of indeterminable type.

Table 19 shows how the types of bacteria were distributed among different subgroups of stillborn and liveborn, and whether they were found in one or more organs. When only one organ was involved, the table shows how often it was the intestine. It also shows how often bacteria were yielded by two or more organs, and how often by two or more organs apart from the intestine.

The table reveals a number of differences between the different groups of infants in types of bacteria. Thus, while coliform bacteria occurred about equally often in the stillborn and liveborn dying within three days, the rate rose in the liveborn dying after the 3rd day.

When only one organ was positive, in the liveborn it was generally a question of coliform bacteria, enterococci and Staph. albus in the intestine, and in the stillborn of these bacteria in the lung.

The nonmacerated stillborn did not differ in any noteworthy respect from the macerated stillborn in types of bacteria.

When two or more organs were infected, the bacteria were generally of the pathogenic variety (coliforms, Listeria, pneumococci, beta-hemolytic streptococci and Staph. aureus). When

bacteria of indeterminate pathogenicity (enterococci, Staph. albus, alpha-hemolytic streptococci) occurred in two or more organs, they were generally mixed with pathogenic bacteria, such as coliforms and Staph. aureus. There were only 4 exceptions to this—1 nonmacerated stillborn infant with only enterococci in the lung and blood, 1 liveborn infant with only Staph. albus in all organs but the blood, and 2 macerated stillborn with a mixed flora of enterococci, Staph. albus and alpha-hemolytic streptococci.

The 31 positive placentas from the autopsied series together yielded 11 kinds of bacteria. Eighteen yielded coliform bacteria. Five (one of which was a monochorionic placenta belonging to a pair of twins) yielded Listeria; in one of these 5 this bacteria was not grown from the fetal organs, and then it only grew when the placental specimens were cultured in enrichment media.

TABLE 17. *Percentage (± standard error) of positive cultures from placentas and fetal membranes in different groups of cases*

	Placenta			Fetal membranes		
	No. cult'd	% pos.	SE of %	No. cult'd	% pos.	SE of %
Total series	113	28	4.2	92	59	5.1
All stillborn	81	25	4.8	65	62	6.0
Nonmacerated and slightly macerated stillborn	46	20	5.9	36	56	8.3
Greatly macerated stillborn	35	37	8.3	29	69	8.7
Liveborn dying within 3 days	32	34	8.5	27	59	9.6
	No group differed significantly from the other			No group differed significantly from the other		

Experiment to study postmortem spread of bacteria from lung and intestine to other organs

A special experimental study was done to get an idea whether bacteria spread from the lung and intestine to other organs after death.

Material and methods. Altogether 13 dead infants, not included in the main series, were used for the experiment, 4 of them liveborn and 9 stillborn. Three of the stillborn were not macerated, 3 slightly macerated, and 3 greatly macerated.

Enteropathogenic E. coli (serological type 0111:B_4) were cultured in dextrose broth. The cultures were incubated for about four hours at +37 C, after which the number of bacteria in them were counted. The bacteria showed good motility in a U-tube (Vahlne, 1945).

TABLE 18. *Stillborn and liveborn subjects by type of bacteria grown from them*

Figures refer to cases, not to organs, and no consideration is given to whether the organism was found alone or combined with others.

Type of bacteria	All stillborn (N=36)	All liveborn (N=76)	Total series (N=112)	
			no.	%
Coliform bacteria				
Colon-aerogenes group	16	53	69	63
Klebsiella pneumoniae	1	—	1	
Alcaligenes fecalis	1	—	1	1
Proteus	1	1	2	2
Pseudomonas aeruginosa	—	6	6	5
Salmonella	—	1	1	1
Hemophilus vaginalis	—	2	2	2
Listeria monocytogenes	3	4	7	6
Pneumococci	—	1	1	1
Streptococci				
alpha-hemolytic	4	5	9	8
beta-hemolytic	2	1	3	3
gamma-hemolytic	—	1	1	1
anaerobic	1	1	2	2
Enterococci	6	20	26	24
Staphylococcus aureus	2	20	22	20
Staphylococcus albus	5	22	27	24
Diphtheroid bacteria	2	1	3	3
Lactobacilli	1	—	1	1
Unidentified gram-negative bacteria	—	1	1	1

The bacterial broth was then injected into the infants. In 7 it was injected into the trachea with a long coarse needle inserted percutaneously down in the larynx, with the body held upright. In the other 6, it was injected into the intestine through a long, narrow rubber catheter, pushed up into the rectum and colon. The rectal temperatures were taken before the injections.

The bodies were then kept first at room temperature (+20 to 22 C), and afterwards in a cold temperature (+4 to +6 C). As only a few subjects were available for this study, large doses of the bacteria were given to them all. A long time was allowed to elapse between inoculation and bacteriological culture so as to get conditions like those in the cases with the longest interval before autopsy in the main series.

Specimens were taken from the organs the same way as in the main series. Immediately after the body was opened, samples were taken from the pleural surfaces and peritoneum (three different places on the surface of the large intestine, and the surfaces of the spleen and liver).

The specimens were cultured on Endo agar plates, both directly and after enrichment in dextrose broth. The serolo-

TABLE 19. *Positive cases in different groups by type of bacteria yielded, and by number of organs in which it was found*

Figures in round brackets denote number of cases in which respective organism was only found in the intestine, in square brackets the number in which it was found in two or more organs apart from intestine.

Type of bacteria	Nonmacerated stillborn (N=16)		Macerated stillborn (N=20)		Liveborn dying within 3 days (N=37)		Liveborn dying after 3rd day (N=39)	
	Only 1 organ	≧ 2 organs	Only 1 organ	≧ 2 organs	Only 1 organ	≧ 2 organs	Only 1 organ	≧ 2 organs
Coliform bacteria								
Colon-aerogenes group	5 (1)	2 [1]	3 (1)	6 [5]	8 (6)	10 [7]	11 (9)	24 [21]
Klebsiella pneumoniae	1	-	-	-	-	-	-	-
Alcaligenes fecalis	-	-	-	1 [0]	-	-	-	-
Proteus	1	-	-	-	-	-	1 (1)	-
Pseudomonas aeruginosa	-	-	-	-	2 (2)	-	3 (2)	1 [1]
Salmonella	-	-	-	-	1 (1)	-	-	-
Hemophilus vaginalis	-	-	-	-	2	-	-	-
Listeria monocytogenes	-	-	-	3 [3]	2	2 [2]	-	-
Pneumococci	-	-	-	-	-	1 [1]	-	-
Streptococci								
alpha-hemolytic	-	-	3	1 [1]	3 (1)	-	2	-
beta-hemolytic	-	2 [2]	-	-	-	1 [1]	-	-
gamma-hemolytic	-	-	-	-	1	-	-	-
anaerobic	1	-	-	-	1	-	-	-
Enterococci	1 (0)	1 [1]	2 (1)	2 [1]	7 (6)	3 [1]	5 (5)	5 [3]
Staphylococcus aureus	1	-	1	-	4 (1)	1 [1]	7 (3)	8 [5]
Staphylococcus albus	2	-	2	1 [1]	8 (6)	3 [1]	8 (6)	3 [1]
Diphtheroid bacteria	2	-	-	-	-	-	1 (1)	-
Lactobacilli	1	-	-	-	-	-	-	-
Unidentified gram-negative bacteria	-	-	-	-	1	-	-	-

gical types of the coliform bacteria in the cultures were checked. When, as in 2 cases, the serological reactions were negative, specimens of the cultures were tested with fluorescein-conjugated, anti E. coli 0111:B_4 globulin according to the method of Danielsson, Laurell & Sjölin (1965). The same method was

Fig. 22

a

b

Fig. 22

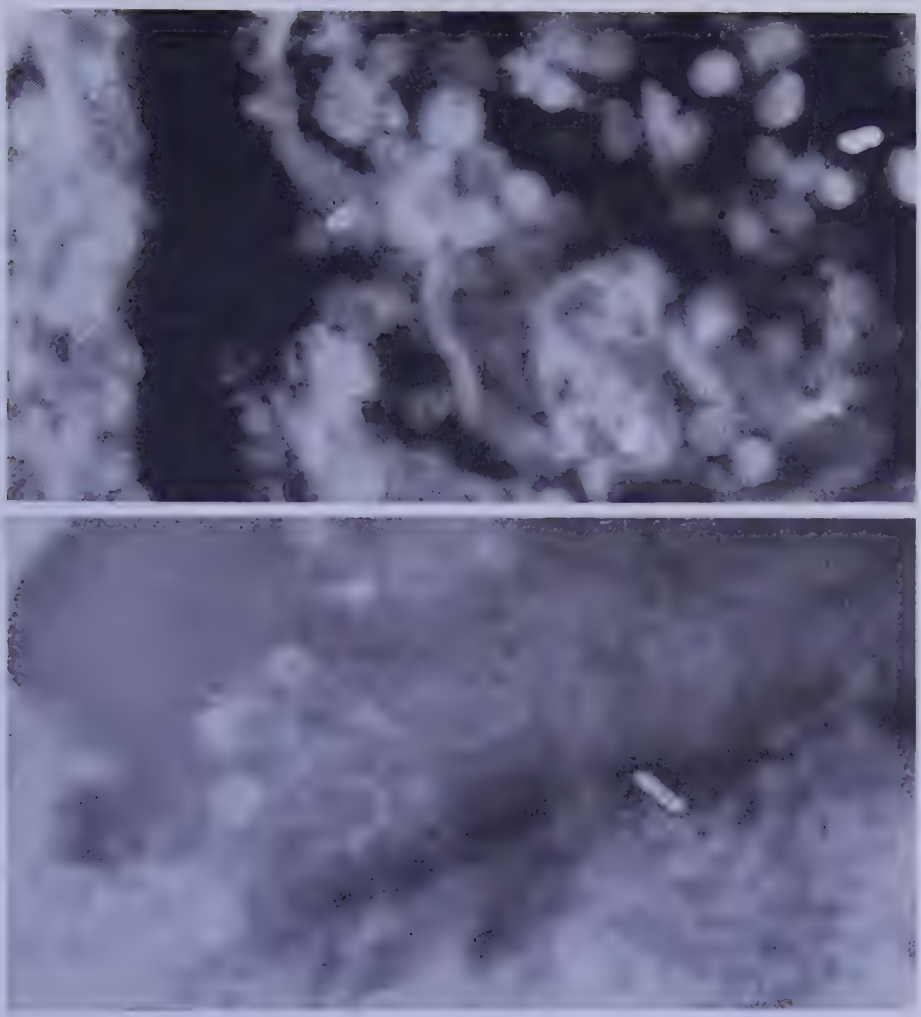

Fig. 22. Postmortem intravascular spread of enteropathogenic E. colli (0111) from lung (case 12 in table 21). (*a*) *Rods in alveolus.* (*b*) *Rod in lumen of branch of pulmonary artery on level with lung hilus.* (*c*) *Three rods in lumen of inferior vena cava.* (*d*) *Rod in lumen of small liver vein. Bacteria visualized with specific fluorescent antibodies* (*with rods in figures b—d slightly retouched*). × *ca. 1,800.*

used for staining the histological sections except that lissamine rhodamine B (RB 200)-conjugated anti Staph. aureus 193 globulin was added to the anti E. coli conjugate (Danielsson, 1965).

Results. The results of these experiments are summarized in tables 20 and 21.

As seen there, in no case, not even of the macerated infants, was it detected

TABLE 20. *Observations on spread of enteropathogenic E. colli (0111:B4) inoculated into intestine (per rectum) after death*

+, test bacteria yielded without prior enrichment; (+), test bacteria yielded only after prior enrichment; 0, no test bacteria yielded by culture; —, no culture done.

	Stillborn non-macerated Case 1	Stillborn non-macerated Case 2	Stillborn slightly macerated Case 3	Stillborn slightly macerated Case 4	Stillborn greatly macerated Case 5	Liveborn Case 6
Weight	2,100 g	3.170 g	2,720 g	840 g	1,330 g	2,500 g
Body temperature before inoculation	27 C	23 C	23 C	24 C	24 C	26 C
Amount bacteria inoculated per ml; no. of ml in brackets	5.6×10^5 (3.5)	1.6×10^9 (4)	4×10^8 (3)	9.2×10^8 (1.5)	8×10^8 (3.5)	1.2×10^9 (2)
Length of exposure after inocultaion to temp.'s in order given:						
+ 20 C	14 hrs.	16 hrs.	11 hrs.	12 hrs.	16 hrs.	11 hrs.
+ 4 C	72 hrs.	48 hrs.	60 hrs.	60 hrs.	50 hrs.	60 hrs.
Results of culture						
Pleura	—	—	—	—	—	—
Lungs	0	0	0	0	0	0
Blood	0	0	0	0	0	0
Five sites on peritoneum	0	0	0	0	0	0
Liver	0	0	0	0	0	0
Spleen	0	0	0	0	0	0
Kidney	0	0	0	0	0	0
Intestine	+[a]	+	+	+	+	+

[a] test bacteria only demonstrable with fluorescent antibodies

that the test bacteria spread from the intestine to other organs.

In 5 out of the 7 cases (1 liveborn, 1 nonmacerated stillborn and 3 macerated stillborn) in which the bacteria were injected into the trachea, they spread from the lungs to the cardiac blood. In 1 case they spread to the liver, but otherwise the cultures from the abdominal organs were negative both in direct cultures and after enrichment. In all except 1 of the cases in which the bacteria spread to the cardiac blood, cultures from the surface of the pleura also yielded the bacteria.

The spread via the blood vessels from the lung to the heart and liver is illustrated in figure 22.

In 2 cases in which the bacteria were injected into the trachea, they spread to the intestine, probably directly from the larynx via the digestive tube.

In 1 case (case 1) in which coliform bacteria were found in the intestine, and in another (case 13), in which they were found in the lung, serological typing of the direct cultures were negative, but testing enriched cultures with fluorescent specific antibodies revealed that some of the bacteria in the cultures were

TABLE 21. *Observations on spread of enteropathogenic E. coli (0111:B4) inoculated into trachea after death*

+, test bacteria yielded without prior enrichment; (+) test bacteria yielded only after prior enrichment; 0, no test bacteria yielded by culture; —, no culture done.

	Stillborn non-macerated Case 7	Stillborn slightly macerated Case 8	Stillborn greatly macerated Case 9	Stillborn greatly macerated Case 10	Liveborn Case 11	Liveborn Case 12	Liveborn Case 13
Weight	2,680 g	3,100 g	1,370 g	875 g	600 g	1,900 g	3,300 g
Body temperature before inoculation	28 C	25 C	26 C	26 C	27 C	27 C	26 C
Amount bacteria inoculated per ml; no. of ml in brackets	1.2×10^9 (2)	9.6×10^8 (1.5)	8×10^8 (1.5)	5.8×10^5 (1)	1.6×10^9 (0.5)	5.4×10^5 (1)	4.4×10^5 (1.5)
Length of exposure after inoculation to temp.'s in order given:							
+ 20 C	12 hrs.	15 hrs.	12 hrs.	13 hrs.	15 hrs.	12 hrs.	12 hrs.
+ 4 C	72 hrs.	55 hrs.	50 hrs.	54 hrs.	9 hrs.	48 hrs.	24 hrs.
Results of culture							
Pleura on both sides	0	+	+	+	0	+	0
Lungs	+	+	+	+	+	+	+[a]
Blood	(+)	+	+	+	0	+	0
Peritoneum	—	—	—	—	—	—	—
Liver	0	0	0	0	0	+	0
Spleen	0	0	0	0	0	0	0
Kidney	0	0	—	—	0	0	0
Intestine	0	+	0	+	0	0	0

[a], test bacteria only demonstrable with fluorescent antibodies

of the serological type injected. It is probable, therefore, that coliform bacteria were present in these two organs before the inoculation. In neither case were coliform bacteria grown from the peritoneal or pleural membranes or other organs.

None of the experimental infants lending themselves to morphological examination showed any morphological signs of infection.

Discussion

Were the bacteria found representative of the bacterial flora in the dead body? In view of the aseptic method of removing the specimens, it is unlikely that they were contaminated with bacteria picked up while they were being removed. This is borne out by the large number of stillborn or neonatally dead subjects who gave negative cultures. That living bacteria in the bodies would not be detected is hardly likely because several organs of each subject were examined, and several different media were used for culturing.

Were the bacteria found in the postmortem cultures representative of the bacteria present while the subject was alive? Clinical reference data, particularly for blood cultures, with which to compare the results of postmortem culture are lacking, especially for the cases of stillbirth and death shortly after delivery. But an attempt was made to answer this question indirectly, by studying the factors which might have caused a difference between the flora present during life and after death.

With increasing lengths of time (up to 72 hours) between death or stillbirth and autopsy, the number of positive organs giving positive cultures increased in general, but only significantly ($P < 0.05$) in the case of the intestine in the stillborn (fig. 1 and 2, table 15). Burn (1934) made the corresponding observation on study of autopsy material from dead subjects of all ages. In the present study, the mean number of positive cultures per subject also increased with the length of time between death or stillbirth and autopsy, but only significantly with the duration between stillbirth and autopsy (table 16). The significant increase for the stillborn also disappeared when allowance was made for the difference in the proportion of macerated and nonmacerated in the different interval groups. From this it would appear that the length of time elapsing before autopsy did not affect the results of the present study to any great degree. The figures for the stillborn, however, were more uncertain statistically than those for the liveborn because of the small number of positive cultures they yielded.

The slight rise in the frequency of positive cultures with the passage of time before autopsy may have been due to the bacteria having multiplied *in situ* after death. It was not possible to make a close study of this question from the results in the present investigation.

A more important question was whether the bacteria spread from one organ to another after death. The experimental study demonstrated that they *can* spread from the lung to the heart

after death, and perhaps to other organs by way of the blood. On the other hand, it indicated that bacteria are not so apt to spread from the intestine to other organs after death. These observations agree in general with those of Burn (1934), who inoculated dead rabbits and guinea pigs with bacteria *per os* or *per rectum;* he noted bacterial spread in only 1 out of 16 animals, however.

The results of the experiment just described, being based on a small series, on only one type of bacteria and on too little variety in bacterial dose and lapse of time before culture, do not allow statistical comparison with the results in the main series.

Statistical analysis of the results from the main series revealed significantly fewer positive cultures from the blood than from the lung in each group (and the same was true of the spleen, liver and kidney compared with the lung in the stillborn and liveborn dying within three days; table 12). It is conceivable that the bacteria not only spread from the lung to the blood, but also from the blood to the lung. Furthermore, several modes of spread are conceivable: from an infection incurred during life, spread during the death agony, and spread after death with or without foci of infection established during life. It is not likely, therefore, that only bacterial spread *after death* without foci of infection established during life had an important effect on the results of culture in the main series.

Of importance in this connection is that the bacteria which spread in the body were nearly always of the pathogenic variety (table 19).

The cases treated with antibiotics yielded a weakly positive lower frequency of positive cultures from the blood from the liveborn infants, but not from other organs (table 14). Another question is how much the treatment with antibiotics affected the bacterial flora *during life.* To this the present study gives no answer.

What organs are best for revealing bacterial infection in autopsy material? In view of the major part played by the lung during the perinatal period, this organ should always be cultured. No significant differences emerged between the blood, spleen and liver on statistical analysis but, as pointed out earlier in this chapter, it is hard to draw definite conclusions from this. Furthermore, it is doubtful whether one can rely on blood cultures alone, because of the possibility of postmortem spread to the blood, especially if the cultures are made a long time after death, and also because of the significantly lower ($P < 0.05$) percentage of positive blood cultures noted in the present study in the cases treated with antibiotics.

It is true that Simmonds (1904), Giordano & Barnes (1922), Hunt *et al.* (1929), and others demonstrated that the results of blood culture agree well with postmortem observations on inflammation, and signs and symptoms of infection during life, as well as with blood cultures during life. But they based their conclusions on cases in which at the most 24—36 hours had elapsed between death and culture. As Canavan & Southard (1915) demonstrated, the

frequency of positive blood cultures begins to increase 12 hours after death. Wood *et al.* (1965) found that a positive correlation between postmortem blood cultures, on the one hand, and morphological lesions and blood cultures during life, on the other, dropped from 83 to 64 per cent 15 hours after death.

A better answer to this question is obtained by comparing morphological and bacteriological data (see chap. 7).

CHAPTER 6

RELATIONSHIP BETWEEN MORPHOLOGICAL AND BACTERIOLOGICAL RESULTS

Relationship between morphological lesions in the lungs and results of culture

The relationship was studied between, on the one hand, the morphological changes in the lungs taken up in chapter 4 and, on the other, the finding of bacteria of any kind and the finding of pathogenic bacteria. Two cases of the granulomatous type of pneumonia were excluded when the correlations were calculated, as this type seems only to occur in listeric infection.

Significance testing. Sixfold tables were used for testing the significance of the correlations found, as some morphological variables were divided into three classes— e.g., massive or moderate prenatal aspiration, slight prenatal aspiration, and no prenatal aspiration. The sixfold tables were converted into three fourfold tables, in the way previously described (p. 38), and each fourfold table was subjected to chi square analysis.

Only once did the three fourfold tables give different results on chi square analysis—on comparison between the occurrence of pneumonia and positive lungs in the stillborn (see under Results).

Results

The correlations between morphological lesions in the lungs and the results of lung culture are seen in tables 22 and 23. There were not enough subjects for each of the morphological lesions in the lungs to make it worth while with statistical analysis of correlations between combinations of these lesions and the results of culture. Nor do the tables take up the results of cultures from other organs, or the occurrence of inflammatory foci in other organs than the lung. But the observations of note in these connections are included in the following.

No correlation of significance was found between prenatal aspiration and positive lung cultures (table 22). Massive or moderate prenatal aspiration was negatively correlated with positive lung cultures, but not significantly; 5 of the 29 stillborn and 1 of the 15 liveborn dying within three days showing massive or moderate aspiration had positive lungs, against 3 of the 10 stillborn and 8 of the 16 liveborn showing signs of slight aspiration, and 9 of the 33 stillborn and 17 of the 70 liveborn showing no morphological signs of prenatal aspiration.

TABLE 22. *Percentage of subjects with various morphological lesions in lungs in cases of positive and of negative lung cultures. Stillborn subjects (N=72) and liveborn dying within three days (N=101)*

Figures in brackets denote number of subjects with positive or negative lung cultures in the respective group. *, ** and *** denote significance at the 5, 1 and 0.1 per cent level, respectively.

Morphological lesion in lung		Results of lung culture: For bacteria of any kind Pos.	Neg.[b]	Diff.	For pathogenic[a] bacteria Pos.	Neg.[c]	Diff.
Stillborn, not or only slightly macerated							
Prenatal aspiration							
Of any degree	%	47	56	—9	42	57	—15
	(N)	(17)	(55)		(12)	(60)	
Massive or moderate	%	29	44	—15	25	43	—18
	(N)	(17)	(55)		(12)	(60)	
Pneumonia							
Diffuse or patchy	%	29	11	18	33	12	21
	(N)	(17)	(55)		(12)	(60)	
Diffuse	%	24	0	24*	25	2	23*
	(N)	(17)	(55)		(12)	(60)	
Interstitial inflammation	%	35	20	15	33	22	11
	(N)	(17)	(55)		(12)	(60)	
Hemorrhage							
Extensive interstitial	%	18	27	—9	17	27	—10
	(N)	(17)	(55)		(12)	(60)	
Diffuse intra-alvoelar	%	6	0	6	8	0	8
	(N)	(17)	(55)		(12)	(60)	
Liveborn dying within 3 days							
Prenatal aspiration							
Of any degree	%	35	29	6	47	28	19
	(N)	(26)	(75)		(17)	(84)	
Massive or moderate	%	4	19	—15	7	16	—9
	(N)	(26)	(75)		(17)	(84)	
Pneumonia[d]							
Diffuse or patchy	%	33	27	6	40	27	13
	(N)	(24)	(75)		(15)	(84)	
Diffuse	%	4	11	—7	7	10	—3
	(N)	(24)	(75)		(15)	(84)	
Interstitial inflammation[d]	%	29	32	—3	40	30	10
	(N)	(24)	(75)		(15)	(84)	
Hemorrhage							
Extensive interstitial	%	12	11	1	7	12	—5
	(N)	(26)	(75)		(17)	(84)	
Diffuse intra-alveolar	%	4	4	0	7	3	4
	(N)	(26)	(75)		(17)	(84)	
Hyaline membranes	%	27	31	—4	13	30	—17
	(N)	(26)	(75)		(17)	(84)	

[a] See p. 19 for bacteria designated as pathogenic
[b] No bacteria of any kind yielded
[c] No bacteria of any kind yielded, or ones of indeterminate pathogenicity
[d] Two cases of listeriosis with granulomatous pneumonia excluded

TABLE 23. *Percentage of subjects with various morphological lesions in lungs in cases of positive and of negative lung cultures. Liveborn subjects dying during 4th to 28th day of life (N=39)*

Figures in brackets denote number of subjects with positive or negative lung cultures in the respective group. *, ** and *** denote significance at the 5, 1 and 0,1 per cent level, respectively.

Morphological lesion in lung		Results of lung culture: For bacteria of any kind			For pathogenic[a] bacteria		
		Pos.	Neg.[b]	Diff.	Pos.	Neg.[c]	Diff.
Pneumonia	%	46	8	38*	44	19	25
	(N)	(26)	(13)		(23)	(16)	
Interstitial inflammation	%	62	54	8	61	56	5
	(N)	(26)	(13)		(23)	(16)	
Hemorrhage							
Extensive interstitial	%	12	8	4	13	6	7
	(N)	(26)	(13)		(23)	(16)	
Diffuse intra-alveolar	%	8	23	—15	9	19	—10
	(N)	(26)	(13)		(23)	(16)	
Macrophages in air spaces	%	39	69	—30	35	69	—34
	(N)	(26)	(13)		(23)	(16)	

[a] See p. 19 for bacteria designated as pathogenic
[b] No bacteria of any kind yielded
[c] No bacteria of any kind yielded, or ones of indeterminate pathogenicity

In none of the cases of prenatal aspiration in which the lung cultures were negative were any bacteria seen in sections of the lung stained for bacteria. Likewise, the cultures from the blood and parenchymatous abdominal organs were negative in all except one of these cases.

To be able to judge the risk of bacterial colonization of the lung in the event of prenatal aspiration, one would have to know exactly what bacteria, if any, were present in the amniotic fluid and delivery passages. As no specimens were taken from these regions in the present investigation, this question can only be answered indirectly by examining the results of culture from the placenta and/or fetal membranes.

Table 24 shows the relationship between the results of culture of the fetal membranes and placenta, on the one hand, and of the lung, on the other, in the cases of massive or moderate aspiration, cases of slight aspiration, and cases with no morphological signs of prenatal aspiration. Only cases in which both the fetal membranes and placenta were cultured are included in this analysis, i.e., about half the cases of prenatal aspiration. Judging by the results shown in table 24, when the fetal membranes or placentas are positive, the risk of bacteria in the lungs is not greater in the cases of massive aspiration than in the ones of no prenatal aspiration.

The rarity of positive lung cultures in the cases of massive or moderate pre-

TABLE 24. *Sixty-four stillborn and liveborn dying within three days with positive or negative cultures from fetal membranes or placenta and positive lung cultures, by different degrees of prenatal aspiration*

Figures in square brackets denote number of stillborn only
By positive lung cultures is meant growth of the same kind of bacteria as in fetal membranes or the placenta

Signs of prenatal aspiration	Culture from membranes or placenta	Cases with positive lung cultures/ total number of cases with positive (or negative) membranes or placenta
Massive or moderate	pos.	2/12 [2/9]
	neg.	0/8 [0/7]
Slight	pos.	1/8 [0/3]
	neg.	1/4 [0/1]
None	pos.	7/16 [4/6]
	neg.	0/16 [0/10]

natal aspiration was apparently not caused by the mother or child getting antibiotic or chemotherapeutic treatment. In only 4 out of the 29 cases of stillbirth with signs of massive or moderate aspiration was treatment of this kind given. It was given in 6 cases of livebirth in which the infants showed signs of massive or moderate prenatal aspiration, and the lung cultures were negative in these 6. In only 1 out of the 9 cases of livebirth with signs of massive or moderate prenatal aspiration not getting treatment of this kind was the lung culture positive.

The diffuse type of pneumonia was positively correlated with positive lung cultures (all types of bacteria) in the stillborn, but the correlation was statistically significant ($P < 0.05$) only when the cases of patchy pneumonia were excluded, or transferred to the cases without pneumonia. Likewise, a significant correlation ($P < 0.05$) was obtained between diffuse pneumonia and pathogenic bacteria in the lungs only if cases of bacteria of indeterminate pathogenicity were pooled with the ones of negative lung cultures. The 4 stillborn infants with diffuse pneumonia all had bacteria in their lungs. In 3 of these cases the bacteria were pathogenic, and the same kind of bacteria were found in several other organs: coliform bacteria in 1 case, coliform bacteria and Staph. aureus in 1 case, and Listeria in 1 case (without granulomas in the lung). The 4th infant showed bacteria of indeterminate pathogenicity in the lung (A. fecalis), but not in the blood or parenchymatous abdominal organs. All these 4 cases of pneumonia showed only slight signs of prenatal aspiration, if any. On the other hand, all showed some degree of interstitial inflammation in the lung.

Only 1 out of the 7 stillborn with the patchy form of pneumonia had positive lung cultures (Staph. aureus and enterococci). Cultures of the blood and parenchymatous abdominal organs were negative in this case, which showed signs of massive prenatal aspiration but not of interstitial inflammation.

Twelve out of the 61 stillborn without pneumonia had positive lung cultures. Eight cases yielded pathogenic bacteria, and in 3 out of the 8 the bacteria were also present in other organs than the lung (beta-hemolytic streptococci in 2 cases, coliform bacteria in 1 case).

In the liveborn dying within three days the correlation between pneumonia and positive lung cultures was weaker than in the stillborn, and not significant (table 22). A weak, nonsignificant, negative correlation was observed between the diffuse type and positive lung cultures. The cultures were positive in only 1 out of the 9 liveborn with this type (pneumococci in the lung and liver); this case showed no signs of prenatal aspiration, but interstitial inflammation was noted. One infant which also showed a diffuse intra-alveolar hemorrhage (hemorrhagic pneumonia) yielded negative cultures from both the lung and the blood and parenchymatous abdominal organs. In the remaining 7 cases the diffuse pneumonia was combined with signs of massive or moderate prenatal aspiration, and in these cases the cultures from both the lungs and the blood and parenchymatous organs were negative.

Seven out of the 19 liveborn infants dying within three days with patchy pneumonia had positive lung cultures. These 7 showed only slight signs of prenatal aspiration, if any. Five of them yielded pathogenic organisms from both the lung and other organs: coliform bacteria in 4 cases, beta-hemolytic streptococci in 1 case. These 5 showed interstitial inflammation in the lung. In 1 case, besides the pneumonia, inflammatory foci were observed in other organs (omphalitis, peritonitis and meningitis). In 2 cases, the lung, but no other organs, yielded bacteria of indeterminate pathogenicity (alpha- and gamma-hemolytic streptococci). Neither of these showed any interstitial inflammation.

Sixteen out of the 71 liveborn dying within three days who did not have pneumonia yielded positive lung cultures. In 9 cases the bacteria were pathogenic, and in 4 of the 9 the same bacteria were present in other organs. Inflammatory foci in other sites than the lung were observed in 2 of these 4 cases: peritonitis in 1 case and lethal epidermolysis bullosa in 1.

The liveborn dying between the 4th and 28th day of life showed only a weakly significant positive correlation ($P < 0.05$) between pneumonia and positive lung cultures. Thirteen of them had pneumonia. Of these, 12 had positive lung cultures. Ten of these 12 yielded pathogenic bacteria (7 coliforms, 1 Staph. aureus, and 2 a combination of coliforms and Staph. aureus). In 7 out of these 10 the bacteria were also found in other organs, and in 3 inflammatory foci were found in other organs besides the lung. The remaining 2 of the 12 infants with positive lung cultures yielded bacteria of indeterminate patho-

genicity (enterococci in 1 case and alpha-hemolytic streptococci in the other).

All 13 infants dying between the 4th and 28th day who had pneumonia showed interstitial inflammation in the lung, mainly characterized by perivasculitis affecting the small interstitial vessels. Septic pulmonary infarction was also seen in 1 case.

Twenty-six of the infants dying at this stage did not have pneumonia. Of these, 14 gave positive cultures from the lung, 13 of them yielding pathogenic bacteria. Eleven of these 13 also yielded bacteria from other organs. In 9 of these 11, inflammatory foci were seen in other organs than the lung (meningitis, peritonitis, enterocolitis or pyelonephritis)

Giving antibiotics or chemotherapeutics to the mother or child apparently had little or no effect on the outcome of postmortem cultures of the lung in the cases of pneumonia. Taking first the group with pneumonia and positive lung cultures, drugs of this kind were given in 4 out of the 5 cases of stillbirth, in 4 out of the 8 of death within three days after livebirth, and in 10 out of the 12 of death after the 3rd day of life. Taking next the group with pneumonia and negative lung cultures, drugs of this kind were given in 2 out of the 6 cases of stillbirth, 7 out of the 21 of death within three days after livebirth, and the 1 case of death after the 3rd day of life.

No correlation of significance was found between interstitial inflammation in the lung and positive lung cultures in any group of infants. But in all except 3 of the cases in which pneumonia was associated with positive lungs there was some degree of interstitial inflammation; most of these yielded pathogenic bacteria from two or more organs. The main type of interstitial inflammation in these cases seemed to be perivasculitis affecting the small blood vessels. As pointed out in chapter 4, the inflammation was often of mild character, only visible on careful microscopic examination of the lung.

No significant correlation, either positive or negative, was observed in any group of infants between extensive interstitial pulmonary hemorrhage and positive lung cultures.

The same was true of diffuse intraalveolar pulmonary hemorrhage. The only stillborn infant with this type of hemorrhage yielded beta-hemolytic streptococci from the lung, spleen and kidney. This infant weighed only 600 g. It did not have pneumonia. One of the 4 liveborn infants dying within three days after birth with a diffuse lung hemorrhage had positive lung cultures. It also showed purulent peritonitis, and coliform bacteria in the blood and parenchymatous abdominal organs. Two of the 5 infants dying between the 4th and 28th day of life who had a diffuse lung hemorrhage yielded positive lung cultures (coliform bacteria and Staph. aureus). One of these also showed purulent meningitis and the same bacteria in several other organs; the other also had pneumonia (hemorrhagic pneumonia).

Thus altogether 4 of the 9 liveborn with diffuse lung hemorrhage gave positive lung cultures.

The correlation between macrophages in the alveoli and positive lung cultures seemed to be negative rather than positive in the liveborn dying between the 4th and 28th day of life. Ten of the 19 of these infants with macrophages had positive lung cultures, and 7 of these 10 also had pneumonia (table 23).

Hyaline membranes were not significantly correlated with positive lung cultures, either positively or negatively (table 22). Only 7 of the 26 infants with this morphological lesion had positive lungs, 2 of them yielding pathogenic bacteria. Only in 1 of the 3 cases in which hyaline membranes were combined with patchy pneumonia was the lung culture positive (child of diabetic mother).

Relationship between placentitis and results of lung culture, between placentitis combined with pneumonia and results of lung culture, and between results of cultures from the placenta, fetal membranes and lung

The morphological and bacteriological observations in the placentas and fetal membranes were compared with the results of the lung cultures on the basis of data from the nonmacerated and only slightly macerated stillborn and the liveborn dying within three days, all born in Uppsala. The different types of pneumonia were pooled for these analyses.

Testing significance. Fourfold tables were used for testing the significance of the correlations between the figures given in table 25. The following is an example.

Placentitis cases

		Lung cultures	
		+	—
Placenta cultures	+	*a*	*b*
	—	*c*	*d*

In this table, *a* stands for the number of cases yielding the same bacteria (whether of one or more kinds) from the placenta and the lung, *b* the number of cases in which the placenta but not the lung yielded bacteria, *c* the number of cases in which the lung but not the placenta yielded bacteria, and *d* the number of cases in which neither the placenta nor the lung yielded bacteria. The cases (2 in number) in which the placenta and lung yielded different kinds of bacteria are put in both *b* and *c*.

When the correlation between placentitis plus pneumonia and the results of lung culture was studied, cases without placentitis or pneumonia, or only one of these lesions, were put in *c* or *d*, depending on whether the lung cultures were positive or negative.

In table 25 the percentages given for positive lung cultures are equal to 100 $a/(a+c)$, and for negative lung cultures to 100 $b/(b+d)$.

The tables were subjected to chi square analysis, and correlations significant at the 5, 1 and 0.1 per cent level were noted.

Results

Table 25 shows whenever a correlation of statistical significance was noted.

There was a positive connection between placentitis and positive lung cultures. But the correlation was significant ($P < 0.05$) only in the stillborn infants, and then only for pathogenic bacteria.

In the 40 cases of placentitis in the stillborn and liveborn in which the lung cultures were negative, the cultures from the blood and parenchymatous abdominal organs were also negative—except for 1 case in which only the kidney yielded bacteria, and 1 in which only the spleen was positive.

When cases of only incipient placentitis were put together with the cases of no placentitis, a weakly significant correlation ($P < 0.05$) appeared between full-blown placentitis and bacteria of any kind in the lungs in the stillborn infants, and the correlation between placentitis and pathogenic bacteria in the lung grew stronger, P falling to < 0.01. But regrouping this way made no difference to the correlation for stillborn plus liveborn, which continued to remain nonsignificant.

The connection between placentitis plus pneumonia and positive lung cultures was slightly stronger in the stillborn than in the stillborn and liveborn combined, but it was not statistically significant. It made no difference if incipient placentitis was transferred to no placentitis. (Nine of the 11 stillborn children with pneumonia were used for this analysis, the placentas being missing for the other 2, both of whom gave negative cultures from the lungs and other organs. Sixteen of the 30 liveborn infants with pneumonia were used for this analysis, the placentas being missing for the other 14. Six of these 14 gave positive cultures from the lungs.)

Placentitis grew more strongly correlated with positive lung cultures when the results of the placenta cultures were taken into account. Bacteria were grown from 15 of the 36 cultured placentas showing placentitis. In 8 of the 15, the same kind of bacteria were grown from the placenta and the lung. These bacteria were all of the pathogenic variety.

Positive placentas, regardless of their morphological condition, were also significantly correlated with positive lungs ($P < 0.01$). Closer analysis shows however, that this correlation was dependent on the cases of placentitis, placentitis being present in 8 of the 9 cases in which the same bacteria were grown from the placenta and lung. In all 8 cases the bacteria were of the pathogenic variety and were grown from several organs.

Positive fetal membranes were significantly correlated ($P < 0.01$) with positive lungs only for pathogenic bacteria.

It is not possible to determine exactly how the number of missing placentas affected the results of the correlation analyses shown in table 25. It is hardly likely that they did so to any great extent. Thus there was no great difference between the proportion of positive and negative lungs in the cases in which the placenta was, or was not examined morphologically (24 against 27 per cent for all kinds of bacteria, and 17 against 16 per cent for only pathogenic ones). Furthermore, as discussed in chapter 4, it is probable that placentitis was about equally common in the placentas not

TABLE 25. *Percentage of subjects with placentitis, with placentitis combined with pneumonia, and with positive cultures from placenta or fetal membranes, in cases of positive lung cultures and in cases of negative lung cultures*

Figures in brackets denote the number of subjects with positive and negative lung cultures in the respective group

*, **, and *** denote significance at the 5, 1 and 0.1 per cent level, respectively

		Results of lung culture					
		Bacteria of any kind			Pathogenic[a] bacteria		
		Pos.	Neg.[b]	Diff.	Pos.	Neg.[c]	Diff.
Placentitis							
Stillborn and liveborn, 0—3 days	%	55	43	12	67	42	25
	(N)	(29)	(93)		(21)	(101)	
Only stillborn	%	71	43	28	83	43	40*
	(N)	(17)	(49)		(12)	(54)	
Placentitis and pneumonia							
Stillborn and liveborn, 0—3 days	%	28	16	12	33	16	17
	(N)	(29)	(93)		(21)	(101)	
Only stillborn	%	29	6	23	33	7	26
	(N)	(17)	(49)		(12)	(54)	
Placentitis and positive cultures from placenta							
Stillborn and liveborn, 0—3 days	%	89	26	63**	89	17	72***
	(N)	(9)	(27)		(9)	(27)	
Only stillborn	%	83	12	71*	83	6	77**
	(N)	(6)	(17)		(6)	(17)	
Placentitis and positive cultures from fetal membranes							
Stillborn and liveborn, 0—3 days	%	88	58	30	88	29	59**
	(N)	(8)	(24)		(8)	(24)	
Only stillborn	%	100	53	47	100	13	87**
	(N)	(5)	(15)		(5)	(15)	
No placentitis, but positive placenta cultures							
Stillborn and liveborn, 0—3 days	%	14	11	3	0	16	—16
	(N)	(7)	(35)		(4)	(38)	
Only stillborn	%	25	5	20	0	14	—14
	(N)	(4)	(19)		(2)	(21)	
No placentitis, but positive fetal membranes							
Stillborn and liveborn, 0—3 days	%	66	39	27	0	20	—20
	(N)	(3)	(28)		(1)	(30)	
Only stillborn	%	100	27	73	0	19	—19
	(N)	(1)	(15)		(0)	(16)	
Positive cultures from placenta (regardless of morphological changes)							
Stillborn and liveborn, 0—3 days	%	56	18	38**	62	15	47**
	(N)	(16)	(62)		(13)	(65)	
Only stillborn	%	60	8	52**	63	12	51**
	(N)	(10)	(36)		(8)	(38)	
Positive cultures from fetal membranes (regardless of morphological changes)							
Stillborn and liveborn, 0—3 days	%	82	48	34	78	24	54**
	(N)	(11)	(52)		(9)	(54)	
Only stillborn	%	100	40	60*	100	16	84***
	(N)	(6)	(30)		(5)	(31)	

[a] See page 19 for bacteria designated as pathogenic

[b] Lung cultures negative or yielding another type of bacteria than the placenta or fetal membranes

[c] Lung cultures negative or yielding bacteria of indeterminate pathogenicity or of another type than the placenta or fetal membranes

examined morphologically as it was in the ones examined morphologically.

The difference between the percentage of positive lungs in cases in which the placenta was, and was not, examined bacteriologically, also amounted to only a few per cent, and no difference of significance was noted.

Morphological lesions in other organs and results of culture

The morphological lesions, inflammation in particular, observed in other organs in the cases of bacterial infection are described in the following. The correlations between the morphological and bacteriological findings are not analyzed statistically for these organs.

Spleen. In most of the cases in which the results of culture and the morphological examination of other organs pointed to bacterial infection, the slides from the spleen only showed hyperemia. Potter (1961) had the same experience in cases of septicemia. Only in 1 case of a stillborn infant with necrosis and granulomas caused by listeriosis, was

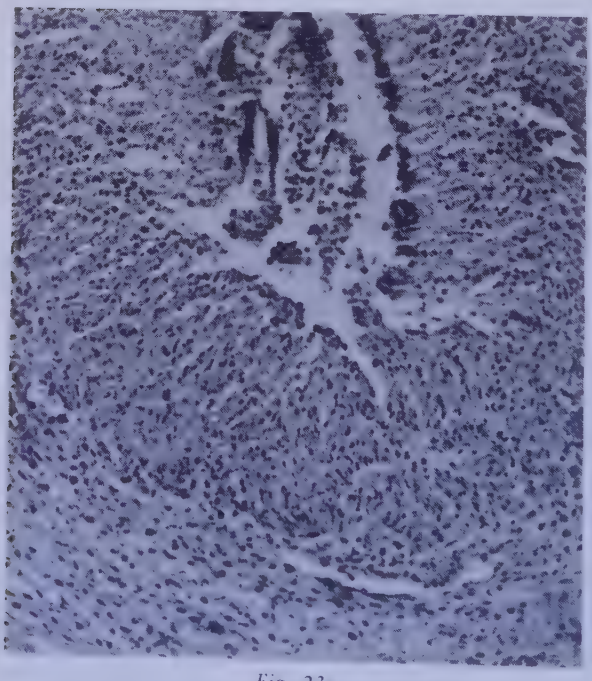

Fig. 23a

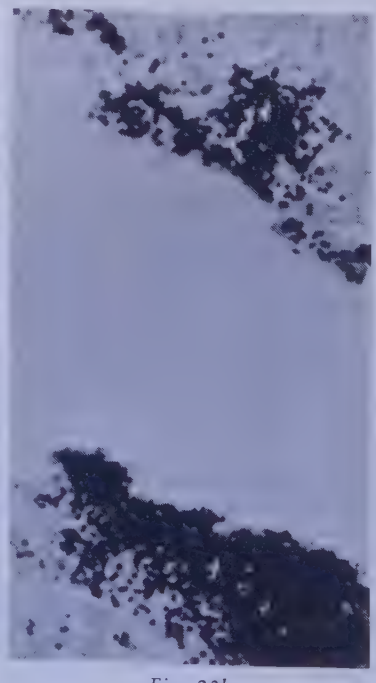

Fig. 23b

Fig. 23. Esophagitis. (*Case 18, table 26*). (*a*) *Detail of esophagus showing widespread ulceration of mucous membrane and infiltration with leukocytes. H & E.,* × *120.* (*b*) *Detail of same case showing accumulations of gram-positive cocci in mucous membrane of esophagus. Gram,* × *900.*

there convincing evidence of inflammation due to bacterial infection. No abscesses were seen in the spleens.

It is known that the spleen is sometimes enlarged in neonatal infection, especially septicemia (Nyhan & Fousek, 1958, and others). The present series contained several cases with enlarged spleens in which, despite bacteriological indications of bacterial infection, the enlargement could be explained in other ways (e.g., Rh incompatibility, severe heart defect). Consequently it is hard, on the basis of the present series, to judge the significance of enlargement of the spleen to bacterial infection.

Liver. Necrosis and granulomas were seen in the liver in 3 cases of generalized infection with Listeria (1 stillborn and 2 liveborn). In the other cases of bacterial infection, the histological picture of the liver was hard to interpret. As in the case of the spleen, it is difficult to say from the present series how strongly enlargement of the liver points to bacterial infection, for in several of the infected cases other reasons could be found for the enlarged liver, such as Rh incompatibility and severe heart defects. No abscesses were seen in the livers.

Esophagus. It is beyond the scope of this publication to go into the origin of esophagitis. But it is interesting to see how often esophagitis was combined with generalized bacterial infection.

By esophagitis is meant here, as in previous studies, ulceration of the mucous membrane of the esophagus and infiltration of leukocytes in the membrane. No culture were made of the esophagus.

Esophagitis was not found in any of the 33 stillborn in whom the esophagus was examined histologically.

It was present in 19 (26 per cent) of the 74 liveborn dying within three days whose esophagus was examined histologically. One of these 19 had listeriosis with granulomas in the esophagus, as well as in the lungs, liver and intestine. In the other 18, the cultures from the blood, spleen, liver and kidney were negative and no foci of inflammation were seen in the other organs.

The 27 liveborn dying within three days whose esophagus was not examined histologically contained only 2 cases of generalized bacterial infection. In both these cases inflammatory foci were found in other organs: pneumonia in 1 and lethal epidermolysis bullosa in 1.

In only 17 of the liveborn dying between the 4th and 28th day was the esophagus examined histologically. Nine of these showed esophagitis. In 2 of these the cultures from the blood and other organs were negative. In the other 7, bacteria were found in several organs; in 4 of these inflammatory foci were found in other organs (pneumonia, peritonitis, pyelonephritis or meningitis). Two infants had been operated on for atresia of the esophagus, and it is hard in these two to decide whether the esophagitis was present before the operation or resulted from it.

One of the 9 infants dying at this stage showed advanced purulent esophagitis without any deformity; Staph. aureus and coliform bacteria were found in the lung, spleen and kidney, and sections from the esophagus also showed

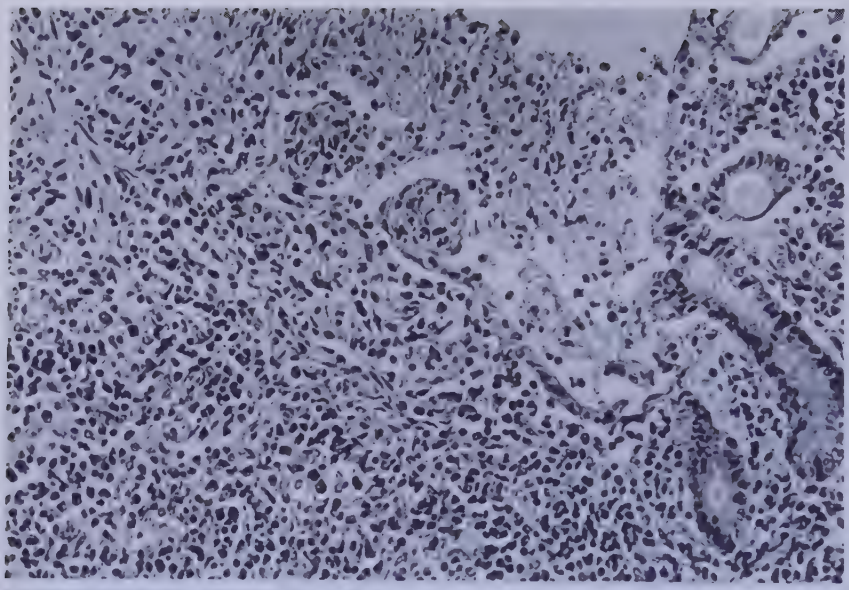

Fig. 24

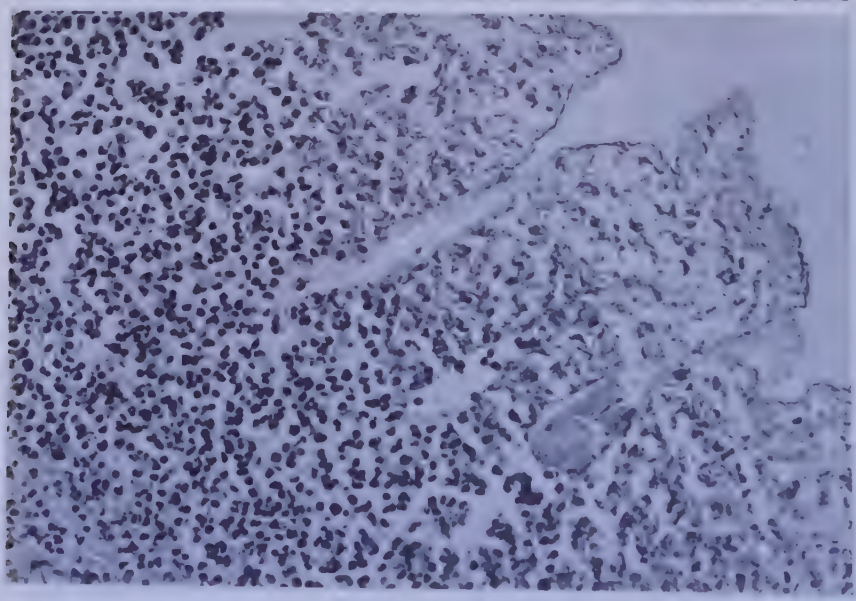

Fig. 25

Fig. 24. Granulomatous enteritis with extensive ulceration in the intestinal mucosa in case of listeric infection. (Case 10, table 26) Van Gieson, × 150.

Fig. 25. Enteritis. Profuse infiltration with leukocytes in mucous membrane. The membranes were also eroded in places. Infection with enteropathogenic E. coli (O127). (Case 34, table 26) H & E., × 150.

a profusion of gram-positive bacteria (fig. 23); as no other inflammatory foci were seen in this case, it is not impossible that the infection started in the esophagus.

Bacteria were found in several organs in 11 of the 23 infants dying after the 3rd day whose esophagus was not examined histologically. In 9 of these, clear foci of inflammation were found in other organs, in the other 2 none.

Intestine and peritoneum. The following lesions were classed as enteritis or colitis: (1) Ulceration and granulomas (fig 24). These were only observed in cases of listeriosis. (2) Ulceration or erosion combined with profuse infiltration of leukocytes into the intestinal mucosa (fig. 25).

Peritonitis, characterized by a fibrinous exudate and abundant leukocytes, was seen in 7 cases.

Kidneys. Acute pyelonephritis with large abscesses were seen in 3 infants, all dying after the first week of life. Two of these also had severely deformed kidneys.

Adrenal glands. One infant yielding coliforms only from the lung showed massive hemorrhage of the adrenal glands (child of diabetic mother). But none of the other infected infants showed this form of hemorrhage.

Brain. Purulent meningitis was observed in 5 cases. In these the exudate contained more mononuclear than polymorphonuclear leukocytes. The brain and spinal cord were only examined microscopically on special indications (chap. 3).

Skin. The series contained 1 case of lethal epidermolysis bullosa. The infant died on the 2nd day from a generalized infection with Staph. aureus.

There was 1 case of purulent omphalitis, also characterized by peritonitis and meningitis. The umbilicus of the infant was not systematically examined histologically.

Discussion

Correlations noted between different abnormalities in an autopsy series, while they may reflect the true conditions in the series in question, are not necessarily valid for the population of both living and dead infants from which it comes. The correlations may be due to the operation of bias. For example, in an autopsy series two abnormal conditions, each a cause of death or the sign of a fatal condition, and each independent of the other, may be negatively correlated, but in the background population of both living and dead infants they may not be correlated, or even be positively correlated. Berkson (1946) pointed out essentially the same thing. On the other hand, if two abnormalities of this kind are positively correlated in an autopsy series, particularly if the correlation is statistically significant, it can not be due to the series being biased, unless it is the very combination of the abnormalities that is fatal.

Several of the morphological lesions studied in this investigation could be a cause of death, or a sign of such a cause: for example, signs of intense prenatal aspiration, extensive intra-alveolar or interstitial pulmonary hemorrhage, and hyaline membranes.

Generally speaking, it is hard to say how much the bacteria found in the different organs had to do with the death of the infants. It should be possible to conclude, however, that pathogenic bacteria, especially if they occur in several organs, were at least a contributory cause of death.

One cannot tell from statistical analysis alone whether or not there is a *causal* connection between morphological lesions and the presence of bacteria. But the statistical results obtained in this investigation, together with the details presented previously in this chapter and with the reports in the literature, give a certain amount of information on what morphological lesions may be considered a sign of bacterial infection, as follows:

(1) In the present series, prenatal aspiration of matter from amniotic fluid or delivery passages was not, as one would have expected, combined with a significantly large frequency of positive cultures (table 22). This was apparently because of the negative connection in the series between intense prenatal aspiration and bacteria in the lung, though the correlation was not statistically significant. It may have been due to bias. In such a case, firstly, the large quantities of aspirated material and the bacteria must have each been a cause of death and, secondly, their presence in the lung must have been purely coincidental. That there was a true negative connection here, however, is indicated by the fact that it was about equally strong in both the stillborn and the liveborn dying within three days, and that intense prenatal aspiration did not seem to incur a larger risk of bacteria in the lung than did no prenatal aspiration (table 24). Nor did the negative correlation appear to be the result of treatment with antibiotics or chemotherapeutics.

This would indicate that large quantities of inspired matter have a bactericide action or inhibit the growth of bacteria. But it is impossible to study this question closer with the present material.

(2) Pneumonia in the stillborn and liveborn dying within three days is not a sure sign of bacterial infection. The same has been noted by other authors (Johnson & Meyer, 1925; Helwig, 1933; Warwick, 1934; Macgregor, 1939; Cruickshank, 1949). Only 13 of the 39 infants with pneumonia in these two groups in the present series had positive lungs (the 2 cases with the granulomatous type occurring in listeriosis not included in the 39). On the other hand, a connection was noted in these infants between placentitis and pneumonia and, in the liveborn at least, also between placentitis and prenatal aspiration, and between prenatal aspiration and pneumonia (table 7 and 8). It is probable, therefore, that pneumonia before the 4th day of life most often consists of inspired amniotic exudate or of an inflammatory reaction to aspirated matter. It is also conceivable that the pneumonia in these cases is sometimes caused by microorganisms, such as fungi or Mycoplasma, not studied in the present investigation.

It is impossible to draw any general conclusions from the present series as to whether the diffuse or patchy type of pneumonia is the most trustworthy

sign of bacterial infection. It is true that the diffuse type was weakly significantly correlated with positive lungs in the stillborn (table 22), and that this correlation was borne out by the positive lungs in all the stillborn with this type of pneumonia. But this correlation is only based on 4 cases. In the liveborn dying within three days, the same correlation was weakly negative, perhaps because in these infants this type of pneumonia usually occurred when there was intense prenatal aspiration, and intense prenatal aspiration, judging by the present results, seems to militate against bacteria in the lung (see foregoing). But here, again, the correlation observed may have been due to the operation of bias. Thus, if one assumes that prenatal aspiration and bacteria can each produce pneumonia, that each may be a cause of death, and that they exist independently of one another, one would not be surprised to find in a biased series, such as one of dead infants, only an occasional instance of intense prenatal aspiration and bacteria among the cases of pneumonia.

The connection between pneumonia and interstitial inflammation in the lung has already been treated by other authors (chap. 4). Interstitial inflammation of some degree was observed in most of the pneumonia cases in the present series (table 7). Interstitial inflammation was also significantly correlated with other lesions (placentitis, prenatal aspiration, hyaline membranes). The fact that some degree of interstitial inflammation was noted in all but 3 cases of pneumonia with positive lungs, and that most of these cases yielded convincing bacteriological signs of infection (pathogenic bacteria in several organs) would indicate, however, that pneumonia can only be considered a sign of bacterial infection when some type or degree of interstitial inflammation is also present. The three cases in the present series which did not meet this criterion, all of patchy pneumonia (1 case each of alpha-hemolytic streptococci, gamma-hemolytic streptococci and Staph. aureus only in the lung), were therefore classed as infection in the bacteriological sense only.

On the other hand, it does not seem right to assume that the newborn infant is infected when it only shows interstitial inflammation in the lung, which some authors call "interstitial pneumonia". Morison (1963) said the same. No case of interstitial plasma cell pneumonia (Pneumocystis pneumonia) was observed in the present series.

To sum up, study of the present material has shown that pneumonia, as morphologically defined here, cannot alone be taken as a sign of bacterial infection in the stillborn and liveborn dying within three days. For this positive bacterial results are necessary. It is conceivable that, even when bacteria are found, the pneumonia is not necessarily a sign of infection, for it may only be a parallel phenomenon. But this is still an obscure question. In the present investigation, therefore, pneumonia was regarded as a morphological sign of bacterial infection if bacteria were found in the lung, provided some degree of interstitial inflammation was also present.

(3) Pneumonia in the liveborn dying after the 3rd day, on the other hand, is a reliable sign of bacterial infection, judging by the present series. It is true that these infants showed only a weakly significant correlation between pneumonia and bacteria (table 23), but the correlation was substantiated by three circumstances: only one of the infants with pneumonia had negative lungs; the infants with both pneumonia and positive lungs generally had bacteria spread throughout their bodies; and the infants without pneumonia but with positive lungs generally had distinct foci of infection in other organs than their lungs. In the last-mentioned cases the positive results from the lungs were probably caused by the bacteria spreading to them from foci elsewhere in the body, either during life, or in the death agony, or possibly after death.

(4) Correlational analysis of other morphological lesions in the lungs and the results of culture did not yield any sure criteria of bacterial infection.

Some authors have suggested that diffuse (massive) intra-alveolar hemorrhage could be caused by bacterial infection (Ylppö, 1919; Brown, 1922; Ahvenainen & Call, 1952). The present series contained too few cases of this type of hemorrhage to permit any conclusions in this regard, and it was not taken as a sign of bacterial infection here.

The present results did not indicate that hyaline membranes increase the risk of bacterial infection.

Macrophages in the alveoli were only connected with bacterial infection when pneumonia was also present.

(5) Placentitis alone did not seem to be a reliable sign of bacterial infection in the infant in the present series. It is true that placentitis was weakly significantly correlated with pathogenic bacteria in the lung of the stillborn, slightly more so when cases of incipient placentitis were excluded, but the same did not hold for the combined group of the stillborn and the liveborn dying within three days. That placentitis and bacterial infection in the infant are rarely combined has been demonstrated by Engström & Ivemark (1960), and others.

(6) Placentitis combined with positive cultures from the placenta seemed to be a more reliable sign of bacterial infection in the infant than only placentitis, especially if the placenta yielded pathogenic bacteria (table 25). Admittedly, positive placentas (regardless of morphological condition) were clearly correlated with positive lungs, but closer analysis revealed that this correlation was dependent on the cases of placentitis.

(7) A combination of placentitis and pneumonia, which others have called the "syndrome of infection of the amnion" or "amniotic infection syndrome", cannot alone be taken as a sign of bacterial infection in the infant. Only 8 of the 23 infants with this combination gave positive cultures, and only 7 of the 19 cases of full-blown placentitis. Whether the placentitis itself was caused by bacteria is another question, one which cannot be settled on the basis of the present results.

(8) Examination of the other organs showed only the conventionally accepted morphological signs of bacterial infec-

tion (peritonitis, enterocolitis, meningitis, pyelonephritis).

The spleen, a useful bacteriological indicator of infection at autopsy, only occasionally showed significant morphological lesions in the cases of bacterial infection. The same was true of the liver.

Esophagitis was common in the present series. It was found in 26 per cent of the infants dying within three days, and in 29 per cent of all the liveborn dying within the first month of life whose esophagus was examined (2 cases of operation for atresia of the esophagus excluded when this frequency was reckoned). This frequency agrees well with the observation of Gruenwald & Marsh (1950) who found esophagitis in 21 per cent, or 23 out of 107 infants dying within the first month whose esophagus they examined histologically.

Despite the high frequency of esophagitis, inflammation in this region was rarely combined with generalized bacterial infection, at any rate in the infants dying within the first three days of life.

Judging from this, during the first three days of life esophagitis rarely gives rise to generalized bacterial infection. Whether the same is true in older infants is harder to say. It is true that esophagitis was combined with general bacterial infection in 7 of the 17 older infants in the series, but 2 of them had been operated on for esophageal atresia and 4 of them had distinct foci of inflammation in other organs.

In only 4 of the present cases was esophagitis noted as a morphological sign of infection: in 1 case of listeriosis with granulomatous esophagitis, 1 case of generalized infection but without inflammatory changes in other organs, and in the 2 operated cases.

CHAPTER 7

CASES OF BACTERIAL INFECTION IN PRESENT SERIES AND CLINICAL OBSERVATIONS

Ninety-eight cases were classed as bacterial infection. Altogether 112 subjects yielded bacteria, but 15 of them yielded them only from the intestine, and showed no clinical or morphological signs of infection, and were therefore excluded. To the remaining 97 was added 1 case of Listeria in the placenta only. (Otherwise cases of positive placentas only were not reckoned as cases of bacterial infection.)

As demonstrated in the previous chapter, inflammation of the placenta points to bacterial infection in the child if the placenta also yields bacteria. The placenta was not examined in several cases, however, and it was not cultured in all the cases in which it was examined morphologically. Because of this, a combination of placentitis and positive placenta cultures was not used as an independent criterion of bacterial infection. Whenever this combination occurred, however, it is noted in the following text.

It is beyond the scope of this report to describe all the clinical observations and discuss their connection with bacterial infection. Moreover, about 85 per cent of the subjects in the present series were stillborn or died within three days after birth, and so it was often impossible to make a thorough clinical analysis of the infant from the point of view of infection. Only the following will be taken into account: (1) Clinical and laboratory evidence of focal infection, (2) maternal fever of 38 C or more during or shortly before delivery, whether or not she showed any signs of focal infection, (3) fever in the child of 38 C or more, (4) a palpably enlarged spleen, (5) a distinctly enlarged liver, the lower border being on a level with the umbilical plane, and (6) jaundice.

Cases of infection in the morphological and bacteriological sense

Data from the 38 cases of infection in both the morphological and bacteriological sense are given in table 26. Eighteen of the infants were born prematurely, weighing less than 2,500 g.

Pneumonia. The most common morphological sign of bacterial infection was pneumonia, being present in 23 cases. Seven of these 23 also had other foci

of inflammation. Two infants had the granulomatous type of inflammation associated with listeriosis (case 10 and 11), and 1 showed septic infarction in the lung in addition to pneumonia (case 17).

Placentitis. Full-blown placentitis was noted in the 4 cases of stillbirth showing pneumonia. The placenta was cultured in 2 of these (cases 1 and 4), and yielded the same kind of bacteria as the infant's organs.

The placenta of 6 infants dying within three days of birth was examined morphologically. Placentitis was noted in 4 of these cases. In 3 (cases 10, 11 and 12) the placenta yielded the same kind of bacteria as the fetal organs. In the 4th (case 8) the placenta was not cultured. The 2 placentas showing no inflammation (cases 6 and 14) yielded another kind of bacteria than the infant's organs.

Peritonitis. Purulent peritonitis was seen in 2 liveborn dying within three days. In 1 (case 13) showing omphalitis the infection might have started in the umbilicus. In the other (case 12) the umbilicus was not examined histologically, but it is possible that the infection started in the umbilical cord here, too, for vasculitis was observed in the cord, as well as placentitis, and both the placenta and fetal organs yielded coliform bacteria.

Purulent peritonitis was also observed in 5 liveborn dying after the 3rd day of life. There it was obviously connected with intestinal malformation and operations to correct it (cases 16, 17, 29, 31 and 32).

Skin infection. In 1 infant dying of lethal epidermolysis bullosa 46 hours after birth the infection obviously started in the skin. The infant showed widespread vesiculation when it was delivered, and deep defects in the skin resembling third-degree burns. Postmortem cultures from the skin gave Staph. aureus of the same phage type as grown from the internal organs, but the fetal membranes and placenta yielded only coliform bacteria.

Meningitis. Purulent meningitis was only observed in infants dying after the third day of life. In 3 cases (cases 20, 21 and 36) lumbar puncture and culture of the cerebrospinal fluid during life showed that the meningitis was present while the infant was alive (in case 21, however, the laboratory evidence was not received until shortly before the infant died); in 2 of these (cases 20 and 21) the fluid yielded coliform bacteria and in 1 (case 36) it yielded Staph. aureus of the same phage type as later yielded by postmortem cultures. In cases 30 and 35, in which no lumbar puncture was done, postmortem cultures of the fluid were negative though purulent meningitis was present. As seen from table 26, the meningitis was obviously connected with hydrocephalus in 3 cases. In 1 of the remaining 2 cases (case 20) otitis media was also present.

Esophagitis. Besides 1 case of granulomatous esophagitis (case 10), there were 3 cases of purulent esophagitis. In 1 (case 18), in which the esophagus was not deformed, none of the other organs were inflamed though several of them yielded bacteria. The other 2 cases were operated on for atresia of the esophagus. In 1 of these (case 19), the su-

TABLE 26. *Cases in series of 264 perinatal deaths which showed morphological lesions indicative of bacterial infection and in which cultures yielded bacteria*

(+) in the bacteria columns denotes that two or more organs among the lung, blood, liver, spleen and kidney yielded the bacteria
(m) in the antibiotics column opposite the first two groups of subjects denotes that the drugs were only given to the mother before delivery, (ch) only to the child

[a], not born in Uppsala; [b] enteropathogenic E. coli (0127); [c] complications listed in chapter 7

Case no.	Sex	Birth wt. g	Age	Morphological signs of infection	Bacteria		Antibiotics	Remarks
					Pathogens	Indeterminate pathogenicity		
Stillborn								
1.	M	2,810	—	Pneumonia (diffuse)	coliforms (+)		No	Placentitis; abnormal delivery[c]
2.	M	4,540	—	Pneumonia (diffuse)	Staph.aureus; coliforms (+)		(m)	Maternal fever; placentitis; abnormal delivery[c]
3.	F	3,420	—	Pneumonia (diffuse)		A. faecalis	(m)	Maternal fever; placentitis; abnormal delivery[c]
4.	F	2,720	—	Pneumonia (diffuse); granulomas in abdom. organs	Listeria (+)		(m)	Maternal fever; maternal shock; placentitis
Liveborn dying within 3 days								
5.	F[a]	2,550	4 hrs	Pneumonia (diffuse)	Pneumococci (+)		No	Mild upper resp. infect. in mother; abnormal delivery[c]; placenta not examined
6.	M	3,370	9 hrs	Pneumonia (patchy)	coliforms (+)	Enterococci (+)	No	Maternal diabetes; no placentitis; hyaline membranes
7.	F	1,420	17 hrs	Pneumonia (patchy)	coliforms (+)		(m+ch)	Abnormal delivery[c]; placenta not examined
8.	F	1,330	36 hrs	Pneumonia (patchy)	coliforms (+)	Enterococci	No	Toxemia of preg.; placentitis
9.	F	1,050	2 hrs	Pneumonia (patchy)	beta-hemolyt. streptococci (+)		(m+ch)	Abnormal delivery[c]; placenta not examined
10.	M	2,330	3 hrs	Pneumonia (granulomatous); granulomatous enteritis; granulomas in abdom. organs	Listeria (+)		(m)	Placentitis; intracranial hemorr.

11.	F	610	1 hr	Pneumonia (granulomatous); granulomatous enteritis; granulomas in abdom. organs	Listeria (+)		No	Upper respir. infect. in mother; placentitis; (binovular twin)
12.	M	1,620	53 hrs	Peritonitis	coliforms (+)		No	Diffuse intra-alveolar lung hemorr.; placentitis; jaundice
13.	F[a]	1,240	60 hrs	Omphalitis; peritonitis; meningitis	coliforms (+)		No	Splenomegaly; placenta praevia; jaundice; placenta not examined
14.	M	2,930	46 hrs	Dermatitis (lethal epidermolysis bullosa)	Staph.aureus (+)		(ch)	No placentitis; abnormal delivery[c]
Liveborn dying on 4—7th day								
15.	F	1,110	7 d.	Pneumonia	coliforms		Yes	(Binovular quadruplet)
16.	M[a]	3,430	3.5 d.	Peritonitis	Staph.aureus; coliforms (+)		Yes	Mult. malform. of kidneys & intest. (oper. on)
17.	M	1,770	5.5 d.	Enteritis; peritonitis; septic lung infarct	Staph.aureus (+)		Yes	Meconium ileus (oper. on); jaundice
18.	M[a]	990	3.5 d.	Purulent esophagitis	Staph.aureus; coliforms (+)		Yes	Intracranial hemorr.; maternal fever
19.	F[a]	2,280	6 d.	Purulent esophagitis; mediastinitis; pneumonia; pleural abscess	Staph.aureus (+); coliforms (+)		Yes	Esoph. atresia (oper. on); clin.diagnosed pleurisy
20.	F	2,300	7 d.	Meningitis; otitis med.	coliforms (+)		Yes	Intravital meningitis; jaundice
21.	M	1,710	5 d.	Meningitis	Staph.aureus; coliforms (+)		No	Diff. lung hemorr.; intracranial hemorr.; clin.diagnosed meningitis; jaundice
Liveborn dying on 8—28th day								
22.	F	1,050	14 d.	Pneumonia	coliforms		Yes	— — —
23.	F	2,270	18 d.	Pneumonia	coliforms (+)	alpha-hemolyt. streptococci	No	Cardiac malform.
24.	M	1,010	21 d.	Pneumonia	coliforms (+)	Staph.albus	Yes	— — —
25.	M	1,430	14 d.	Pneumonia	Staph.aureus; coliforms (+)	Ps. aeruginosa	Yes	Diff. lung hemorr.

TABLE 26 (cont.).

Case no.	Sex	Birth wt. g	Age	Morphological signs of infection	Bacteria: Pathogens	Bacteria: Indeterminate pathogenicity	Anti-biotics	Remarks
26.	F[a]	3,210	14 d.	Pneumonia	— — —	alpha-hemolyt. streptococci	Yes	Intracranial hemorr.
27.	M[a]	2,870	27 d.	Pneumonia	coliforms (+)		Yes	Esoph. atresia (oper. on)
28.	F[a]	3,370	11 d.	Pneumonia; esophagitis	coliforms (+)		Yes	Esoph. atresia (oper. on); cardiac malform.; hydramnion
29.	F[a]	4,100	12 d.	Enteritis; peritonitis; pneumonia	Staph.aureus	Enterococci (+); Staph. albus	Yes	Omphalocele (oper. on)
30.	F[a]	4,010	22 d.	Meningitis; pneumonia	coliforms		Yes	Hydrocephalus (Arnold-Chiari); fever
31.	M[a]	2,980	19 d.	Peritonitis	coliforms (+)	Enterococci (+)	No	Mongolism; mult. intest. malform. (oper. on); cardiac malform.
32.	F[a]	2,200	16 d.	Enteritis; peritonitis	Staph.aureus (+); coliforms (+)	Ps. aeruginosa (+); enterococci (+)	Yes	Maternal diabetes; intest. malform. (oper. on); cardiac malform.; hydramnion
33.	F	3,040	9 d.	Enteritis	coliforms (+)		Yes	Hepatomegaly; liver necroses; intracranial hemorr.
34.	M	4,240	24 d.	Enteritis	coliforms (+)[b]		No	Mongolism; megacolon (oper. on); cardiac malform.; fever
35.	M	2,940	21 d.	Meningitis; pyelonephritis	coliforms (+)		No	Hydrocephalus (Arnold-Chiari); clin. diagnosed cystitis
36.	F	2,540	23 d.	Meningitis	Staph.aureus (+); coliforms (+)		No	Hydrocephalus (Arnold-Chiari); clin. diagnosed meningitis
37.	M[a]	2,650	11 d.	Pyelonephritis	Staph.aureus; coliforms (+)		No	Mult. intest. malform. (oper. on); kidney malform.; fever; clin.diagnosed cystitis
38.	M	3,720	20 d.	Pyelonephritis	Staph.aureus; coliforms (+)		Yes	Cystic disease of kidneys, liver & pancreas; clin.diagnosed cystitis; fever

tures gave way after the operation, and mediastinitis and a pleural abscess developed. Cultures of the pleural exudate during life in this case yielded Staph. aureus of the same phage type as found in the postmortem cultures.

Enterocolitis. Enterocolitis was present in 7 cases. Of these, 2 had the granulomatous type of inflammation accompanying listeriosis (cases 10 and 11). Four had intestinal malformations which had been operated on. The remaining infant (case 33) also showed multiple liver necroses of obscure origin, as well as rupture of the tentorium cerebelli and signs of an old subdural hemorrhage. Infection was suspected while the infant was alive, but no focus of infection was found.

Pyelonephritis. Acute pyelonephritis with multiple abscesses was found in 3 infants, all dying after the 7th day of life (cases 35, 37 and 38). Infection of the urinary passages was noted during life in all 3 cases. In case 37, however, the diagnosis was not established until shortly before the infant died.

Bacteria. Coliforms were the organisms most commonly grown, being found alone or combined with other bacteria in 28 of the 38 cases. Only 1 of the coli strains grown belonged to the enteropathogenic variety (case 34).

Staph. aureus was found in 13 cases, in 11 mixed with other bacteria.

Beta-hemolytic streptococci (group F) were isolated from 1 case (case 9).

Pneumococci were found in only 1 case (type 2,4). This infant was born at a hospital near Uppsala and died there. The mother had a mild upper respiratory infection.

Listeriosis was noted in 3 cases (cases 4, 10 and 11). A twin of case 11 was severely macerated and was grouped with the cases of only bacteriological infection; the twins had a common placenta. The Listeria strain was of type 2 in case 10 and of type 1 in case 11. No typing was done in case 4.

In one case (case 4) the mother's temperature rose to 40 C during delivery but fell the next day to 35 C, at the same time as her systolic blood pressure dropped to 80 mm Hg. During and after delivery she had a small vaginal hemorrhage, but she lost only 100 ml.

In none of the 3 cases were Listeria found in the mother's vagina, cervix, urethra, urine or blood after delivery. In case 10 it was noted that the mother's antibody titer to Listeria-O-antigen rose from 1/10 three days after the delivery to 1/80 two weeks later.

As seen from the table, 2 cases yielded only bacteria of indeterminate pathogenicity. In case 3, A. fecalis were found in the lung (and intestine). During the delivery of this infant the mother's temperature rose to 39 C; full-blown placentitis was also observed. The mother had been given antibiotics for four days before delivery, and it may have been that other bacteria besides those found at autopsy had been present before. In case 26, only alpha-hemolytic streptococci were found in the lung, but the infant had got antibiotics during the week before it died, and it may have been that it originally harboured other bacteria.

Fever. Fever was noted in 4 infants, all of whom died after the first week of life. In case 34, 37 and 38, the fever

was of the septic type, but in the 4th (case 30) only one peak to 38 C was noted. In another case (case 29) a temporary rise was noted on the third day, but as this was apparently due to dehydration the fever was not mentioned in table 26.

Enlargement of spleen or liver. Enlargement of the spleen was noted during life in 2 cases (cases 13 and 31). In 1 (case 31) this might have been due to cardiac malformation. Enlargement of the liver was noted during life in cases 33 and 38; in case 38 it was obviously due to cystic disease of the liver (the kidneys and pancreas were also cystic). In 7 cases the hospital records say nothing one way or the other about the size of the spleen and liver.

Jaundice. Jaundice was noted in 5 liveborn infants dying within the first week of life (cases 12, 13, 17, 20 and 21). These 5 accounted for half the infants dying between the 3rd and 7th day of life (table 26). Only in 1 case (case 17) did the serum bilirubin exceed 15 mg per hundred milliliters.

Comparative value of different organs for showing bacterial infection

The question of the value of different organs for showing bacterial infection was taken up in chapter 5. Table 26 throws further light on this question. In 37 out of the 38 cases of infection, the lung was cultured, in 38 the blood, in 37 the spleen, in 34 the kidney, in 21 the liver and in 35 the intestine. Bacteria were yielded by the lungs in 35 cases, or 95 per cent. The corresponding percentages for the other organs were: blood 53 per cent, spleen 65 per cent, kidney 76 per cent, liver 63 per cent, and intestine 91 per cent.

Table 27 takes up the cases with the same type of bacteria in two or more organs, the intestine excluded. The lung was also positive in a large proportion of these—94 per cent. The blood was more seldom positive than the spleen, liver or kidney. The kidney was slightly more often positive than the spleen and the liver.

Cases of infection only in the bacteriological sense

As seen from table 28, 36 infants yielded pathogenic bacteria but showed no morphological signs of infection. A large proportion of them were premature, 25 weighing less than 2,500 g at birth.

Twenty-three of the 36 had only one positive organ; in 14 cases this was the lung.

Seven also yielded bacteria of indeterminate pathogenicity. Only 1 of these, however, yielded bacteria of this kind (enterococci) from more than one organ; this infant also had coliform bacteria in several organs.

Coliform bacteria alone were grown from 23 infants, in 7 cases from two or more organs. None of them were of the enteropathogenic variety. In 1 of the 23, the mother had received antibiotics, and in 6 the child. The placenta was examined morphologically in 15 (either stillborn or liveborn dying within three days) and in 9 of these placentitis was observed. In 1 of these 9, that of a non-

TABLE 27. *Percentage of positive cultures from different organs in cases of infection in both the morphological and bacteriological sense and in which cultures yielded bacteria in two or more organs*

Organ	Cases with both morphological and bacteriological signs of infection	
	No. in which organ pos./no. in which organ cultured	Percentage of pos. cultures from organ
Lung	30/32	94
Blood	20/32	63
Spleen	24/32	75
Liver	13/18	72
Kidney	26/31	84

macerated stillborn infant, coliform bacteria were grown from both the placenta and internal organs. (In 7 of the 9 no cultures were made of the placenta.) The mother of one severely macerated child was feverish during delivery, but in none of the other 22 did either the mother or child have any fever. None of the liveborn in this group had a palpably enlarged spleen. One infant dying the 13th day of life had shown progressive enlargement of the liver, but it also had a severe cardiac defect. Three liveborn dying after the 3rd day of life were jaundiced (2 also showing kernicterus), in 1 case probably due to a liver malformation, in 1 to intracerebral hemorrhage and in 1 (of maternal diabetes) to massive hemorrhage in the adrenal glands and retroperitoneal tissue.

Staph. aureus alone was found in 3 cases, each time in only one organ, (lung, spleen or blood). No antibiotics had been given in these cases. None of the 3 placentas were examined. In 1 liveborn infant the bacteria was found in the blood; this infant showed liver enlargement of possibly infectious origin. In another case, in which the bacteria was found in the spleen and intestine, the intestine also yielded type C Salmonella; the infant, who died on the 27th day of life and had a severe heart defect, showed no signs of infection.

Three cases yielded both Staph. aureus and coliform bacteria. Antibiotics had been given to the mother in 1 case and to the child in 2 cases. The placentas of these cases were not examined. In no case did the mother or liveborn infant have any fever, or the infant show enlargement of the liver or spleen, or jaundice.

Listeria were found in 5 cases. In 2 of these (both of severely macerated stillborn infants) the bacteria grew profusely in cultures from both the placenta and fetal organs, and the histological slides also showed an abundance of Listeria rods on staining with the Gram and Levaditi method. Full-blown placentitis was noted in both cases. One of the infants was the twin of a liveborn infant who died an hour after birth (case 11, table 26); both twins yielded

TABLE 28. *Number of cases in series of 264 perinatal deaths lacking morphological lesions indicative of bacterial infection but yielding pathogenic bacteria*

Figures in brackets denote number of cases in which two or more organs among the lung, blood, liver, spleen and kidney yielded the bacteria in question

Birth weight in grams	Bacteria yielded					Total	Born in Uppsala
	Coliforms	Staph. aureus	Staph. aureus & coliforms	Listeria	Beta-hemol. strept.		
Stillborn, not or only slightly macerated							
501—1,000	1 (1)	-	-	-	1 (1)	2 (2)	2 (2)
1,001—2,500	2 (0)	-	-	-	1 (1)	3 (1)	3 (1)
>2,500	5 (0)	1 (0)	-	-	-	6 (0)	5 (0)
Stillborn, greatly macerated							
501—1,000	2 (1)	-	-	2 (2)	-	4 (3)	4 (3)
1,001—2,500	2 (1)	-	-	-	-	2 (1)	1 (1)
>2,500	1 (1)	-	-	-	-	1 (1)	1 (1)
Liveborn dying within 3 days							
501—1,000	1 (0)	-	1 (0)	3 (0)	-	5 (0)	5 (0)
1,001—2,500	3 (1)	1 (0)	-	-	-	5 (2)	5 (2)
>2,500	1 (0)	-	1 (1)	-	-	1 (0)	1 (0)
Liveborn dying on 4—7th day							
1,001—2,500	3 (1)	-	1 (1)	-	-	4 (2)	2 (1)
>2,500	1 (0)	-	-	-	-	1 (0)	0
Liveborn dying on 8—28th day							
>2,500	1 (1)	1 (0)	-	-	-	2 (1)	1 (1)
Total	23 (7)	3 (0)	3 (2)	5 (2)	2 (2)	36 (13)	30 (12)
Born in Uppsala	19 (6)	1 (0)	3 (2)	5 (2)	2 (2)	-	-

Listeria of type 1. In the other of the 2 cases (a boy weighing 900 g) type 2 were grown. In the remaining 3 infants, all dying during the first three days of life, Listeria were found in only one organ and only after enrichment. In 1 case, only the placenta yielded Listeria (type 2), but it was not inflamed. Listeria were grown from the lung (type 2) in 1 of the remaining 2 cases and from the liver (type 4b) in the other. Neither of these organs showed any signs of inflammation. The mother of one of the infants had shown signs of pyelitis shortly before delivery. In none of the 5 mothers were Listeria found in cultures from the vagina, cervix, urethra, urine or blood after delivery. Nor did they have an elevated antibody titer to Listeria-O-antigen. None of the liveborn infants showed enlargement of the liver or spleen, or jaundice.

Beta-hemolytic streptococci were grown from the placenta and several organs of 2 premature infants, both stillborn but not macerated. In 1 case the strain belonged to group G (type 16); the strain from the other case was not typed. Full-blown placentitis was present in both cases. The mother of one of the infants was feverish during delivery and had a blood-stained vaginal discharge. She had a sore throat for three weeks before the delivery and had taken antibiotics for it. The mother of the second child had a normal temperature during delivery, but got a high fever the day after. She showed no signs of focal infection. One infant had a diffuse intra-alveolar pulmonary hemorrhage, and the other a large intracranial hemorrhage.

Table 29 takes up the cases in which only bacteria of indeterminate pathogenicity were grown from the postmortem cultures. They were 24 in all; only 4 yielded bacteria from more than one organ. Antibiotics had been given in 6 cases; only to the mother in 1 case, to the mother and child in 1 case, and only to the child in 4 cases. The placenta was examined morphologically in 15. Placentitis was noted in 10 cases; in none of these were the same bacteria found in the placenta as in the fetal organs. The mother of 1 child dying 40 hours after birth had cystitis; the child only yielded alpha-hemolytic streptococci from the lung. None of the other mothers showed signs of infection during the delivery or in the days shortly preceding delivery. Enlargement of the spleen and jaundice were observed in 1 child which had erythroblastosis. Enlargement of the liver was noted in a liveborn infant which only yielded enterococci, and only from the lung, but it had a severe heart defect. None of the liveborn of this group had ever been feverish.

Resumé of bacterial infection among Uppsala-born infants

Eleven per cent of the 218 infants born in Uppsala and dying within perinatal period II showed bacterial infection in both the morphological and bacteriological sense. Adding to these the cases of infection only in the bacteriological sense and yielding pathogenic bacteria accounts for 25 per cent of

TABLE 29. *Number of cases, in series of 264 perinatal deaths, with no morphological lesions indicative of bacterial infection in which cultures yielded only bacteria of indeterminate pathogenicity*

Figures in brackets denote number of cases in which two or more organs among the lung, blood, liver, spleen and kidney yielded the bacteria in question

Birth weight in grams	Alpha-hemol. strepto-cocci	Entero-cocci	Staph. albus	Either H. vaginal, anaer. strept., diph-theroids, lacto-bacilli or unident. gram-neg. bacteria	Mixed flora	Total	Born in Uppsala
Stillborn, not or only slightly macerated							
501—1,000	-	-	1 (0)	1 (0)	-	2 (0)	2 (0)
1,001—2,500	-	-	-	2 (0)	-	2 (0)	2 (0)
>2,500	-	1 (1)	-	-	-	1 (1)	1 (1)
Stillborn, greatly macerated							
1,001—2,500	-	1 (0)	-	-	1 (1)	2 (1)	2 (1)
>2,500	1 (0)	-	1 (0)	-	2 (1)	4 (1)	4 (1)
Liveborn dying within 3 days							
501—1,000	-	-	1 (0)	-	-	1 (0)	0
1,001—2,500	1 (0)	1 (0)	3 (0)	4 (0)	1 (0)	10 (0)	6 (0)
Liveborn dying on 4—7th day							
>2,500	-	1 (0)	-	-	-	1 (0)	1 (0)
Liveborn dying on 8—28th day							
>2,500	-	-	1 (1)	-	-	1 (1)	1 (1)
Total	2 (0)	4 (1)	7 (1)	7 (0)	4 (2)	24 (4)	19 (4)

the series. Adding to these again the cases only yielding bacteria of indeterminate pathogenicity accounts for 34 per cent of the series.

The corresponding figures for the 166 infants born in Uppsala and dying within perinatal period I were 9, 20 and 31 per cent.

Complications of pregnancy and delivery, and severe malformations and other severe lesions in the cases of infection

A series of infants dying shortly before and after birth is naturally biased, in containing a large proportion of complications of pregnancy and delivery, as well as of severe malformation and other serious lesions in the child. It is often hard, therefore, to judge how much proven bacterial infections have to do with the deaths in comparison with other abnormalities, and this question lies outside the scope of the present study.

In table 30 are set out the additional complications and lesions occurring in the cases of infection in the present study. By "Complications of pregnancy" in this table is meant: maternal diabetes, toxemia of pregnancy, Rh incompatibility, hydramnion, placenta praevia, or abruptio placentae. By "Abnormal labour and delivery" is meant: premature rupture of membranes (6 hours or more before labour started), 30 hours or more of labour before delivery, abnormal position or presentation of fetus necessitating obstetrical intervention (including breech delivery with manual extraction) or profound neonatal asphyxia leading to attempts at resuscitation. The fetal malformations include severe deformities in the central nervous system, cardiovascular system, lungs and abdominal organs (including meconium ileus). By "other severe lesions" is signified: rupture of the tentorium cerebelli with subdural hemorrhage, intracerebral hemorrhage with blood in the cerebral ventricles, kernicterus, massive pulmonary hemorrhage, or massive hemorrhage in other organs or body cavities, massive prenatal aspiration of particulate matter, hyaline membranes and interstitial pulmonary emphysema with or without pneumothorax.

As seen from table 30, most of the cases of stillbirth and death within three days were complicated in different ways during pregnancy or delivery—47 out of the 65 cases in these groups. When the cases of severe deformity or other severe lesion are added, it leaves only 9 cases in these groups. If the cases of prematurity are added to these, it leaves only 4 cases.

The end results were much the same for the infants dying between the 4th and 28th day. But these infants naturally differed from the two foregoing in containing more cases of fetal deformity and severe lesion than complications of pregnancy and delivery.

Discussion

As evident from the tables in this chapter, 60 out of the 98 cases of infection were classed as infection in the bacteriological sense only. Thirteen of the 60 infants, however, were greatly macerated stillborn who did not lend themselves to histological analysis. Most

TABLE 30. *Various complications and disorders in the 98 out of 264 cases of perinatal death showing bacterial infection*

Complications and disorders listed in chapter 7. Figures in brackets denote number of cases yielding pathogenic bacteria

	Bacterial infection					
	Morphological and bacteriological			Only bacteriological		
	Stillborn N=4	Liveborn 0—3 days N=10	Liveborn 4—28 days N=24	Stillborn N=29(18)	Liveborn 0—3 days N=22(11)	Liveborn 4—28 days N=9(7)
I Complications of pregnancy	0	3	3	8 (5)	10 (4)	2 (2)
II Abnormal labour & delivery	3	4	6	13 (9)	12 (8)	2 (1)
I+II (in no. of subjects)	3	7	7	18 (12)	19 (8)	3 (2)
III Fetal malformations	0	1	15	5 (3)	4 (2)	4 (2)
IV Other severe lesions	0	3	7	3 (3)	11 (6)	3 (3)
III+IV (in no. of subjects)	0	4	20	8 (6)	15 (8)	7 (5)
I+II+III+IV (in no. of subjects)	3	9	22	24 (16)	20 (10)	8 (6)
V Prematurity (birth w't < 2,500 g)	0	6	8	17 (11)	21 (10)	4 (4)
I+II+III+IV+V (in no. of subjects)	3	10	24	26 (16)	22 (11)	9 (7)

of the 60 only yielded bacteria from one organ apart from the intestine—generally the lung. This is not surprising. For one reason, the series contained many cases of complicated deliveries—for instance, premature rupture of the membranes or anoxia necessitating resuscitatory measures—which increase the risk of bacteria entering the respiratory passages. For another, the upper respiratory passages of even the normal newborn often contain bacteria, at least after the first day of life (Smith & Bloomfield, 1950; Laurell, 1952), and the chances of bacteria entering into their lungs may be great, for it is probable that the reflexes guarding their respiratory passages are not completely developed, and if the infant is anoxic, the reflexes may even be depressed (de Carlo *et al.*, 1952; Morison, 1963).

The significance of bacteria in only one organ is hard to evaluate if there are no morphological signs of infection. When bacteria are found in more than one organ, one has more reliable bacteriological support for the presence of bacterial infection. That postmortem spread of bacteria was an essential source of error in the present study seems hardly likely (chap. 5). In addition, bacteria were grown from two or more organs in 32 out of the 38 cases of infection in both the bacteriological and morphological sense (table 26), but in only 17 out of the 60 cases of infection for which morphological signs of infection were lacking (tables 28 and 29); moreover, 7 of these 17 were greatly macerated stillborn whose organs could not be evaluated histologically, and in at least 4 of the 17 there was no doubt that the infection was of clinical significance; 2 of greatly macerated stillborn showing Listeria rods in several organs, and 2 nonmacerated stillborn with beta-hemolytic streptococci in several organs.

Accordingly, judging from the experience accumulated from this study, one should make postmortem cultures from several organs in cases of perinatal death—from at least the lung, blood, spleen and kidney, and preferably the liver and intestine as well. Apparently one cannot rely on cultures of the blood alone (chap. 5 and table 27). It is true that the lung is good for revealing the presence of generalized bacterial infection (table 27), but this organ is more apt to be the only positive organ than are the blood and parenchymatous abdominal organs, especially in cases of stillbirth and death in the first few days of life (table 12).

Coliform bacteria predominated in the 98 cases of infection in the present study, occurring alone or together with other bacteria in about 54 per cent. Many have drawn attention to the high frequency of these and other gram-negative bacteria in cases of neonatal septicemia (e.g., Nyhan & Fousek, 1958; Buetow *et al.*, 1965). During recent years the proportion of coliform bacteria in neonatal septicemia has been increasing, probably because of the use of antibiotics. These bacteria, however, have always been prominent in the bacterial spectrum in the perinatal period. Macgregor, for instance, showed as far back as 1939, thus before the introduction of penicillin and other antibiotics, that the most common bacteria in the lung in perinatal pneumonia belonged to

the B. coli group, these being grown from 43 out of her 106 cases of positive lungs combined with pneumonia.

Staph. aureus was grown in about 19 per cent of the 98 cases. After coliform bacteria, this was the most common bacteria found. But, while the coli infections were distributed about equally over the stillborn and liveborn of different ages, Staph. aureus occurred mainly in the older of the liveborn infants, who were usually handicapped by congenital malformation, and often operated on.

Streptococci and pneumococci only occurred in 3 and 1 per cent of the cases, respectively. Some infants may have harboured these bacteria before they died and had been rid of them by antibiotics, but it is impossible to tell how often this happened. Several authors have noted, however, that these bacteria are growing less and less frequent in cases of neonatal infection. For example, Buetow *et al.* reported recently that they only found 1 case of streptococcic infection, and none of pneumococci, among 158 premature infants with neonatal septicemia revealed by blood cultures while they were alive.

Listeria were found in 8, or about 8 per cent, of the 98 cases. This figure is probably not representative of the frequency of Listeria in cases of perinatal death in the Uppsala region. Thus, the routine bacterial cultures done since 1962, after the material for this study had been collected, have not uncovered any more Listeria.

Two of the cases of listeriosis in this study have already been described (Olding & Philipson, 1960) and all except 1 of the cases (case 4, table 26) are included in an earlier study of listeriosis in the Uppsala region (Ekelund *et al.*, 1962). These cases will therefore not be discussed any further here. Particularly interesting, however, are the three cases in which Listeria were found in only one organ and in which no inflammatory reaction was discerned. It is hard to interpret these cases. No others of this kind in humans seem to have been described before the Uppsala study. Case 4 (not previously reported) is also particularly interesting—the one in which the mother had a high fever during delivery and the next day fell into vascular shock. It was not quite clear what brought on the shock, but the most likely explanation seemed to be the listeriosis in the child. Other authors have described shock in cases of septic abortion (e.g. Deane & Russel, 1960, Stevenson & Yang, 1962; Woodard, 1962; Neuwirth & Friedman, 1963), but none of them mention listeriosis in this connection. Furthermore, as far as I know, no other authors reporting on human listeriosis have described a case in which the mother of an infected infant got a shock of this kind in connection with delivery.

It is impossible to tell exactly how the noncultured Uppsala-born infants affected the figures for bacterial infection in the consecutive Uppsala series. But as these accounted for only 19 per cent of the series and were mostly from short consecutive periods of time during which no cultures were made in cases of perinatal death, it is highly unlikely that they affected the frequencies to any notable degree.

SUMMARY

A study was made of the bacterial infections occurring among infants dying during the perinatal period of life: a postmortem morphological and bacteriological analysis was made of 264 infants autopsied during the four years 1959 to 1962 in Uppsala—123 stillborn and 141 liveborn dying within 28 days after birth, all weighing more than 500 g at birth. Eighty-five per cent were either stillborn or died within the first three days of life. Fifty-five of the stillborn were greatly macerated, and could only be examined bacteriologically. In addition, 164 placentas belonging to 111 stillborn and 56 liveborn dying within three days were examined morphologically, 113 of them bacteriologically as well.

Bacterial infection was defined in two ways: (1) When there were morphological signs of bacterial infection and the postmortem cultures yielded bacteria, the case was classed as infection in both the morphological and bacteriological sense. (2) When postmortem cultures yielded bacteria but morphological evidence of bacterial infection was lacking or impossible to obtain (in greatly macerated stillborn), the case was classed as infection in the bacteriological sense only.

The specimens to be cultured were all removed under strictly aseptic conditions by a routine method. Cultures were made routinely from all or most of six organs: the lung, blood, spleen, liver, kidney and intestine.Eight different culture media were used for each organ, including special enrichment media for Listeria.

The infants yielded 19 types of bacteria. Of these, coliforms, Listeria, Salmonella, beta-hemolytic streptococci, and Staphylococcus aureus were classed as "pathogenic", and the others as "of indeterminate pathogenicity".

The cultures were negative from all the organs in 71 per cent of the cases of stillbirth, and in 60 per cent of the liveborn dying within three days. Twenty-one per cent of the stillborn had positive lungs and 26 per cent of the liveborn dying within three days. The corresponding figures for positive intestines were 13 and 27 per cent. The figures for the blood and parenchymatous abdominal organs varied between 7 and 12 per cent. The two groups of infants differed significantly ($P < 0.05$) only for positive intestines.

Different lapses of time (up to 72 hours) between death or stillbirth and autopsy were not associated with statistically significant differences in percentages of positive cultures from the lungs, blood, spleen, liver or kidneys, or in the average number of positive

organs per infant. (The dead infants were kept in a cold temperature until they were autopsied.) A separate experimental study of dead infants indicated that bacteria may spread from the lung to the blood after death, but analysis revealed that this could not have had a major effect on the results obtained from the organs in the main series. Nor did postmortem spread from the intestine to other organs appear to be a major source of error. The liveborn infants given antibiotics had a weakly significantly ($P < 0.05$) lower percentage of positive postmortem blood cultures than the untreated infants, but did not differ significantly for the other organs. The effect of antibiotics on the bacteria present while the children were alive could not be studied.

Accordingly, the results of culture obtained from the infants after death were probably representative of the bacterial flora in the bodies after death and, as far as it was possible to ascertain indirectly, they were also representative of the bacteria in the bodies before death.

Using the term pneumonia in a wide sense—for all stages from small clumps of leukocytes in occasional alveolar ducts or alveoli to a diffuse dissemination of leukocytes all over the lung—pneumonia was observed in 15 per cent of the stillborn, in 30 per cent of the liveborn dying within three days and in 18—50 per cent of the liveborn dying after the 3rd day. (These include a few cases of granulomatous pneumonia in cases of listeric infection, hemorrhagic pneumonia, and septic lung infarction.) Comparison between the morphological and bacteriological results, however, revealed that pneumonia (except the granulomatous variety) was not a reliable indication of bacterial infection in the stillborn and the liveborn dying within three days after birth. It seems that in infants of this age pneumonia often consists of inspired amniotic exudate or of a reaction to prenatally inspired matter. In the present study pneumonia was taken as a morphological sign of bacterial infection only when the lungs were positive for bacteria and when they also showed some degree of interstitial inflammation. In infants dying between the 4th and 28th day of life pneumonia seemed to be a more reliable sign of bacterial infection.

Signs of intense prenatal aspiration of particulate matter, whether or not associated with pneumonia, seemed to be negatively rather than positively correlated with bacteria in the lung, though this could not be confirmed statistically. It was hard to determine whether there was a connection between diffuse intra-alveolar pulmonary hemorrhage and bacterial infection. Hyaline membranes, judging from the results, do not increase the risk of bacterial infection. No connection was noted between macrophages in the air spaces and the presence of bacteria.

Placentitis, when used to mean inflammation in the placental membranes and umbilical cord regardless of degree, was observed in 50 per cent of the stillborn and liveborn dying within three days; the most advanced form (chorioamnionitis, usually combined with vasculitis in the umbilical cord) was observed in 29 per cent of these two groups

of infants. Placentitis in itself was apparently not a reliable sign of bacterial infection in the child. Nor did this seem to be true of placentitis combined with pneumonia, a combination others have called "the amniotic infection syndrome". Placentitis combined with positive placental cultures seemed to be a more reliable sign.

Nothing of note was observed in regard to the morphological signs of infection in the other organs. They showed the conventionally accepted signs, such as purulent meningitis and peritonitis, enterocolitis, pyelonephritis, and omphalitis.

Esophagitis combined with ulceration was seen in 29 per cent of the 91 liveborn infants whose esophagus was examined histologically. Judging by the present series, a generalized bacterial infection rarely starts with esophagitis, at any rate in liveborn infants dying within the first three days after birth.

The results indicated that several organs should be cultured, not only one, in order to get reliable proof of the presence of bacterial infection in the perinatally dying infant, preferably the lung, blood, spleen and kidney.

To sum up the observations on the bacterial infections in the infants examined:

Bacterial infection in both the morphological and bacteriological sense was noted in 38 cases, or 14 per cent. When the cases with no morphological signs of infection but yielding pathogenic bacteria are also classed as infection, the percentage of infected cases rises to 28 per cent. (Adding the cases showing only bacteria of indeterminate pathogenicity and without any morphological signs of infection brings the percentage up to 37.)

Separate analysis of the 218 infants born at the University Hospital (representing about 80 per cent of the Uppsala-born infants dying in Uppsala during the four years in question) reveals that, of the stillborn and liveborn dying within 28 days after birth weighing more than 500 g (perinatal period II), 11 per cent showed both morphological and bacteriological signs of infection, and 25 per cent no morphological signs of infection but pathogenic bacteria. (Adding to these the cases only yielding bacteria of indeterminate pathogenicity and no morphological signs of infection gives a percentage of 34.) The corresponding figures for the Uppsala-born stillborn and liveborn dying within the first week of life weighing more than 1,000 g (perinatal period I) were 9 and 20 per cent (and 31 per cent).

Coliform bacteria were grown from 54 per cent of the 98 infected infants, and these were the most common bacteria in each group of infants; only one of the E.coli strains isolated was of the enteropathogenic variety. Staph. aureus was yielded by 19 per cent; it was most common in the older liveborn infants. Listeria was grown in 8 per cent. (This figure is probably not representative for the occurrence of listeriosis among perinatal deaths in Uppsala, for no more cases have been uncovered since the ones for this study were assembled.) In 3 cases the Listeria only appeared in special enrichment media, and in only one organ, and the bacteria were not accompanied by morphological signs of

infection. The mother of a stillborn infant with a listeric infection had a high fever during delivery, and the next day got a vascular shock. Beta-hemolytic streptococci were isolated from 3 per cent, and pneumococci from 1 per cent.

Focal infection had been diagnosed during life in 7 of the 98 infected infants; all 7 died after the 3rd day. Fever was noted in 4 infants, all dying after the 7th day.

Eighty-six of the 98 infected infants were handicapped by: complications of pregnancy or delivery, or severe malformation, or other severe organic lesions. When the cases of prematurity are added to these 86, only 4 cases are left.

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Appendix

STATISTICAL METHODS

Notation

Mean: $\bar{x} = \frac{\sum_{i=1}^{N} x_i}{N}$, where x_i denotes the i'th recorded value in a series of N values.

Relative frequency:

$$p = \frac{\text{number of incidences}}{N}$$

Standard deviation:

$$SD = \sqrt{\frac{\sum(x_i - \bar{x})^2}{N-1}}$$

Standard error of the mean (SE):

$$SE = \frac{SD}{\sqrt{N}}$$

in comparison between two groups:

$$SE_{\bar{x}-\bar{y}} = \sqrt{\frac{\sum (x_i - \bar{x})^2}{(N_x - 1)N_x} + \frac{\sum (y_i - \bar{y})^2}{(N_y - 1)N_y}}$$

Standard error of a relative frequency:

$$SE = \sqrt{\frac{p(1-p)}{N}},$$

standard error of difference between two relative frequencies or percentages:

$$SE_{p_1 - p_2} = \sqrt{\frac{p_1(1-p_1)}{N_1} + \frac{p_2(1-p_2)}{N_2}}$$

Standard weighted percentages:

$$\sum W_j P_j,$$

where P_j is the percentage in the j'th sub-group,
W_j is the weight of the j'th sub-group, and

$$\sum W_j = 1.$$

The weights, W_j, were chosen to correspond approximately to the number of subjects in the respective sub-groups.

Significance tests

The following significance test were used:

1. In percentage differences: *chi square test* usually with one degree of freedom. Yates' correction was applied (pp. 38, 63, 69).
2. In mean differences:

$$\frac{\bar{x} - \bar{y}}{SE_{\bar{x}-\bar{y}}}$$

which approximately follows the standardized normal distribution (or t-test if $N < 30$).

3. In differences between standard weighted percentages:

$$\sum W_j \, (P_{xj} - P_{yj})$$

a normally distributed critical ratio is produced:

$$\frac{\sum W_j \, (P_{xj} - P_{yj})}{\sqrt{\sum W_j^2 \frac{N_{xj}P_{xj} + N_{yj}P_{yj}}{N_{xj} + N_{yj}} \left(100 - \frac{N_{xj}P_{xj} + N_{yj}P_{yj}}{N_{xj} + N_{yj}}\right) \left(\frac{1}{N_{xj}} + \frac{1}{N_{yj}}\right)}}$$

where N_{xj} and N_{yj} correspond to the number of subjects in the compared sub-groups (pp. 49, 50).

4. The *sign test* was applied in testing the hypothesis that two sub-groups are of equal size (p. 46).

For references on significance tests see Snedecor (1956) and Hald (1948).

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